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TRANSACTIONS of the BOSE RESEARCH INSTITUTE CALCUTTA

Vol. VIII, 1932-1933
BIOLOGICAL AND PHYSICAL
RESEARCHES

EDITED BY
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WITH 131 ILLUSTRATIONS

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BIOLOGICAL AND PHYSICAL RESEARCHES

I.—INTRODUCTORY

BY

SIR J. C. BOSE, F.R.S.

IN founding the Research Institute, the object has been the pursuit of investigations in different branches of knowledge. The first investigations, which gave important results, were on the quantitative study of the optical properties of electric waves as well as the polarisation of electric radiation by various crystals and the selective conductivity exhibited by the polarising structures. The continuation of these investigations led to the discovery of the phenomenon of response of inorganic matter to stimulus, the response of the living and non-living exhibiting a very remarkable parallelism. The very long series of investigations subsequently carried out in the Institute were on the essential similarity which underlies the physiological mechanism in the plant and in the animal. The scope of research has recently been considerably expanded by including additional subjects of investigation in Zoology and in Anthropology. Special attention is devoted once more to the pursuit of physical investigations in regard to certain recent advances on the subject.

Among the results described in the present volume is the study of possible differential action of water disturbance and of vegetable extracts on different fishes. In regard to the action of disturbance produced in water, though the fishes are devoid of the power of perception of sound, yet there is an indication that some of them might be less

affected by water disturbance than others. Another problem arises for the selective elimination of the fish *Bhetki* which destroys other fishes preserved in tanks. Certain vegetable extracts which have been employed as "fish poisons" cannot be utilised for the purpose since they are found to be very virulent in their action and cause the death of fishes in general. The extract of the plant *Cajanus indicus* is, however, found to be more discriminative in its action. Special investigations carried out on the subject showed that while dilute extract of *Cajanus indicus* did not produce any evil effect on the generality of fishes, it, however, brought about paralysis and death of the *Bhetki*.

The pulsatory fall of the leaflets in *Averrhoa bilimbi* forms the subject of another series of investigations. It has been observed that this occurs during increasing darkness towards the evening. An anomaly is found in the fact that, under excessively strong light, a series of pulsatory falls is produced apparently similar to those due to gathering darkness. Automatic records, obtained by means of special recorders, proved that there is a characteristic difference between the two types of responsive movements. In addition to the above two types, a new kind of pulsatory fall has been discovered which may be distinguished as the specific response to darkness: a very large amplitude with extraordinary rapidity of the responsive fall characterises this new type of response. The leaflets of *Averrhoa* exhibit, moreover, multiple responses not only to photic but also to other modes of intense stimulation. It has been further shown that there is a continuity between multiple and autonomous responses.

An important generalisation has been established by the demonstration that the motor mechanism is essentially similar in sensitive and in ordinary plants. Polar excitation of the plant-tissue by a constant electric current due to Kathode-make and by Anode-break gives rise, as in the animal tissue, to a contractile reaction. In the relatively sluggish and less excitable ordinary plants, two additional reactions have been discovered which are due to Kathode-break and to Anode-make. These induce an expansive or

anti-excitatory reaction. It has also been shown that while transmitted impulse impinging directly on the motor organ induces a contractile fall, the indirect effect of the impulse is of an expansive character, causing erectile movement of the leaflet. The transmission of the impulse in both sensitive and ordinary plants can be repeatedly arrested or restored by the alternate application of the electrotonic block in the same manner as the nervous impulse in the conducting nerve of the animal can be arrested or restored ; this proves the essential similarity of the physiological process of conduction in the plant and in the animal. There is a preferential direction of propagation of the impulse in plants, the velocity in centrifugal direction being considerably greater. In certain cases the difference is so great that the conduction becomes irreciprocal, occurring only in the centrifugal direction. .

The effect of external stimulus on diametric growth of stems has been quantitatively determined by means of automatic recorders specially devised for the purpose. The sensitiveness of the method has been greatly increased by the Method of Balance. The effect of light on diametric growth of thin stems has thus been found to be a retardation of the rate of growth, while the effect of light on a thick stem is a preliminary acceleration, followed by a retardation of the rate. The cause of this difference has been discovered and explained. The suggested explanation has been justified by the observation of the effect of electric stimulation. Under this mode of stimulation, there is no characteristic difference of reactions induced in thin and in thick stems. The effect of light on diametric growth is found to be modified by the age of the specimen. In a vigorously growing internode light induces a retardation of the rate, while in an old internode it causes an acceleration. Light thus induces two opposite effects, depending on the age of the organ. This fact explains the diversity observed in the tropic movements of the stem under unilateral action of light, the response being positive or negative according to the age of the internode on which the light is incident. In cases where the entire length of one side of the stem is exposed to unilateral light, the result

becomes extremely complicated ; for the movement of the apex of the stem is then determined by the algebraical summation of the two different movements, positive or negative, which occur in younger and in older internodes.

A new method has been devised and perfected for the automatic record of the growth of roots and the variation induced in the rate under changed external conditions. This has been rendered possible by utilising the pressure exerted by the growing root upon a suspended aluminium pan of a very sensitive balance. The effect of application of dilute CO_2 on both aerial and underground roots is found to be an enhancement of the rate of growth ; prolonged application induces, however, a retardation of the rate. The effect of a concentrated dose of CO_2 is a preliminary enhancement of the rate followed by a great depression. The after-effect of a previous application of ether is often a very marked enhancement of the rate of growth. The vapour of ammonia exerts a remarkable action on certain underground roots. The immediate effect induced by it is a retardation of the rate of growth ; its continued application, however, brings about an active contraction of the organ. On removal of the vapour of ammonia, the growth of the root becomes slowly revived.

The characteristic effects of various drugs on the rhythmic tissues of the animal and the plant have been studied and the results described.

Experiments carried out on the effects of indigenous Indian drugs show that the effect of each on different sections of the alimentary tract is not the same, but characteristically different. Moreover, while a particular drug may induce a contractile reaction of the organ, another drug might give rise to the opposite reaction of relaxation.

A large number of experiments were carried out with numerous species of *Coniferae* growing in the hills of Darjeeling as well as in the plains. The manganese content of the plant was found to undergo a change according to its habitat. The Himalayan *Coniferae*, whose natural habitat is at a higher level, exhibit a diminution of the manganese content when they are grown in the plains. The peninsular

Coniferae, which grow naturally at lower altitudes, show, on the other hand, a higher manganese content in the plains than when grown in the hills. The manganese content in the leaves of *Coniferae* undergoes a marked increase during production of female flowers; this increase, found in the leaves of flowering branches, is probably the source for the supply of the relatively large amount of manganese which is found in the cones. *Coniferae* which give rise to male inflorescence only do not show any noticeable increase of manganese content in their leaves. In healthy green leaves of certain plants, the manganese content is found to be much higher than what is found in chlorotic leaves of the same plants. This seems to indicate that a sufficient quantity of manganese is one of the factors for proper functioning of the green leaves.

A new organic compound has been isolated from the extract of *Eupatorium Ayapana*. A crystalline substance with a definite melting point has been obtained by repeated crystallisation from special solvents. The molecular formula of the compound is found to be $C_{12}H_{10}O_4$. The characteristic properties of the compound and its reactions under the action of different reagents are given in detail.

Further investigations have been carried out on the oils from leguminous pulses. In *Kabuli chhola oil*, as in some others, there is an indication of the possible presence in it of A and D vitamins.

In the Department of Anthropology, investigations have been carried out on certain Primitive Tribes in India, as represented by the Mundas in the district of Ranchi. The measurements of skulls as well as of living types demonstrate the important fact that while these Aborigenes, generally speaking, agree with the Veddahs and Australians, they nevertheless differ significantly from the Proto-Australoid type of crania found in the prehistoric civilisation of the Indus Valley.

In the Department of Physics investigations have been carried out on characteristic absorption spectra of silver and lead halides vapour. These results are likely to throw much light regarding the photo dissociation of polyatomic molecules.

II.—THE POSSIBILITY OF DIFFERENTIAL EFFECTS ON CERTAIN FISHES BY WATER DISTURBANCE AND BY VEGETABLE EXTRACTS

BY

SIR J. C. BOSE, F.R.S.

FOR the supply of edible fishes, the ponds in Bengal are usually stocked with Rohit (*Rohita bengalensis*), Mrigal (*Cirrhina mrigal*), and Katla (*Catla buchannani*), for which there is a great demand. Of these the rate of growth in *Katla* is considerably quicker, hence it is more profitable to collect them before the others for the market. A question arises: How are these particular fishes to be preferentially caught in the net? There is a prevalent belief amongst the fishermen, that while the fishes in general are frightened away by the throwing of stones or of clumps of earth into the pond, the *Katla* are not much affected by the disturbance, but may even be attracted towards it. In catching the *Katla* fish in preference, the fishermen are thus in the habit of throwing clumps of earth into the pond, after which a circular net is cast over the centre of disturbance. The sinkers attached to the circumference of the net also cause water-disturbance, as they sink to the bottom. It is thought that, by the differential action of the water-disturbance, all other fishes are more or less scared away, while a large proportion of the *Katla* left behind are caught in the net.

THE PERCEPTION OF SOUND

The question arises, whether it is the sound as such or whether it is the water-disturbance that might possibly affect the different fishes in an unequal manner. Since the

fishes do not possess any properly developed organ of hearing, it is not at all likely that they are able to perceive sound, a conclusion which has found general acceptance. It was, however, thought desirable to subject the question to special tests, in order that sound as such could be isolated from mechanical disturbance in the water, caused by the throwing in of clumps of earth.

Experiment 1. *Effect of sound on fishes.*—The experiment was carried out in a large aquarium which contained

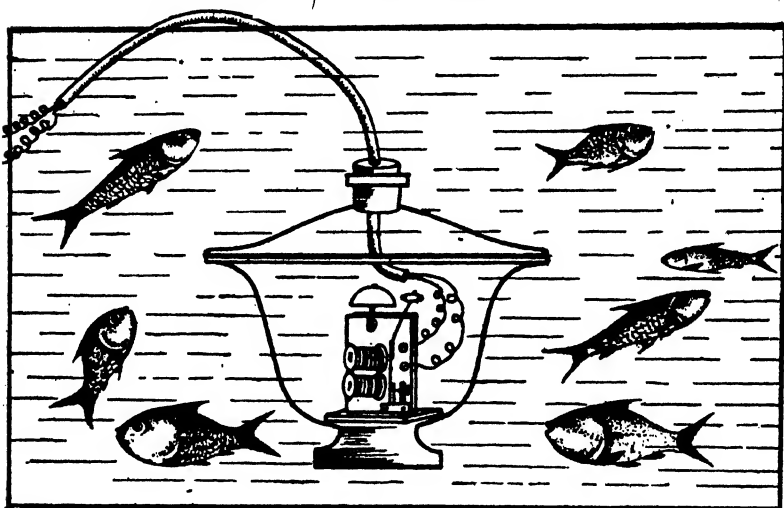


FIG. 1. Method of producing sound-note inside the water without causing any mechanical disturbance.

The sound produced did not have any effect on the different fishes.

a considerable number of different varieties of fish; the glass sides of the aquarium afforded every facility for observation. In order to produce sound without any mechanical disturbance in the water, an electric bell was enclosed in a suitable glass trough, with an indiarubber stopper at the top, through which a rubber tube was led outside. This rubber tube enclosed two insulated wires for connection with a battery; the periodic pressure of a tapping key outside could thus produce successive ringings of the bell inside the water. The object of the precautions taken in encasing

the bell in a glass trough is to prevent the leakage of the current into the water, as well as to prevent short-circuiting of the magnetising coil of the bell by the semi-conducting water, which would render the bell ineffective after a short time.

After carrying out these precautions the electric bell inside the water was repeatedly sounded. The sound-note thus produced did not, however, induce any effect on the fishes, which were neither attracted towards nor frightened away from the source of sound (fig. 1).

Experiment 2. *Effect of a high-pitched note.*—Further investigations were carried out for determining whether a high-pitched note would prove to be more effective. For this particular test a whistle, which gave out a note of high pitch, was sounded time after time, but even this high-pitched note proved to be quite ineffective, neither attracting nor frightening away any of the fishes.

It is, therefore, evident that the fishes examined were incapable of perceiving any sound.

EFFECT OF WATER-DISTURBANCE

Having found that sound as such had no effect on the fish, investigations were next undertaken to find whether water-disturbance produced any differential action on various fishes. The experiments were carried out at different places: (1) in large tanks at the Research Station at Falta on the Ganges; (2) at Dhakuria, a few miles outside Calcutta, and (3) in the experimental tanks at the Bose Research Institute in Calcutta.

In the following investigations certain special precautions had to be taken in order to secure the best results. Many fishes, *Katla* in particular, possess protruding eyes, and their power of seeing is exceptionally acute; they thus become quickly aware of any impending danger from outside the water. But the water in the tanks utilised for stocking the fish is practically opaque in most cases, on account of vegetative growth; this prevents the fishes seeing the persons from whom danger is apprehended. Neither are they able to see the actual fall of the stones or clumps of earth that

may be thrown into the water. A partial opacity of the water in the tank is therefore an important condition in the study of the effect of pure water-disturbance on the fish.

Experiment 3. *Observation at Fatta tank.*—In this large tank both *Katla* and *Rohit* were stocked in about equal numbers. After large clumps of earth had been dropped in the tank, the circular net was thrown so that its centre was just over the origin of maximum disturbance. On drawing the net a very large number of *Katla* was found to have been caught, while the number of *Rohit* was relatively few. Similar results were obtained several times in succession.

Experiment 4. *Observation at Dhakuria.*—Several varieties of edible fish were stocked in this particular tank. On the throwing of the net three times in succession, the total catch was 47 in number, of which 35 were *Katla* while 12 belonged to the other varieties. Here also the water-disturbance did not evidently scare away the *Katla* to the same extent as it did the other fishes.

In the experiments described above, the proportion of the different fishes present in the tanks, though approximately equal, was not exactly known. The next series of observations were taken in a tank in the Research Institute in Calcutta, the exact proportion of the two varieties of fish, *Katla* and *Rohit*, which were introduced into the tank, being definitely known.

Experiment 5. *First series of observations in the tank of the Calcutta Institute.*—In the present case 9 *Katla* and 126 *Rohit* had been introduced into the tank. The density of distribution of two varieties of the fish was thus in the proportion of 1 *Katla* to 14 *Rohit*, symbolised by the formula $K_1 : R_{14}$. From this it would naturally follow that, in the case of no differential action induced by water-disturbance, the number of the two different species of fish caught at each throw of the net would be as $K_1 : R_{14}$.

The following gives the results obtained with four successive throws of the net. The fishes caught were thrown back into the tank, the relative proportion of the two fishes being thus kept constant.

At the first throw 1 *Katla* was caught and only 1 *Rohit*, the proportion being $K_1 : R_1$ instead of $K_1 : R_{14}$.

At the second throw also 1 *Katla* was caught and 1 *Rohit*, the proportion being once more $K_1 : R_1$ instead of $K_1 : R_{14}$.

At the third throw 2 *Katla* were caught and no *Rohit*, the proportion being $K_2 : R_0$ instead of $K_1 : R_{14}$.

At the fourth throw the number of *Katla* caught was 4 and no *Rohit*, the proportion being $K_4 : R_0$ instead of $K_1 : R_{14}$.

The next series of experiments were carried out under conditions which were still more favourable to *Rohit* than to *Katla*. This will be understood from the fact that the number of *Rohit* was kept always the same, namely, 126, those caught each time being thrown back into the tank. The *Katla* caught at each successive throw of the series, on the other hand, were taken away, leaving a decreasing number of this particular fish in the tank.

Experiment 6. *Second series of observations in the tank of the Calcutta Institute.*—The proportion of *Katla* in relation to *Rohit* at the beginning was $K_9 : R_{126}$, the density of distribution being $K_1 : R_{14}$. At the first throw of the net 2 *Katla* were caught and 1 *Rohit*, the proportion being $K_2 : R_1$ instead of $K_1 : R_{14}$. On removal of the 2 *Katla* caught, and returning the *Rohit* to the tank, the density of distribution became $K_7 : R_{126}$ or $K_1 : R_{18}$.

At the second throw of the net the number of *Katla* caught was 3, while not one *Rohit* was obtained, the proportion of the catch in the case of the two fishes being $K_3 : R_0$ instead of $K_1 : R_{18}$. After removal of the 3 *Katla*, there were left in the tank only 4 *Katla*, and the density of distribution became $K_4 : R_{126}$ or $K_1 : R_{32}$ approximately.

At the third throw 1 *Katla* was caught and 2 *Rohit*. The proportion of the catch was, therefore, $K_1 : R_2$ instead of $K_1 : R_{32}$.

Though it does not appear that the *Katla* fish was attracted by the disturbance, yet the results of the series of observations that have been described may perhaps give some support to the view that the *Katla* fish is, relatively speaking, less affected by the water-disturbance.

DIFFERENTIAL ACTION OF VEGETABLE EXTRACTS IN
DRUGGING DIFFERENT FISHES

Extracts of certain plants have often been employed as 'fish-poisons,' the effect of which is first to paralyse and then cause the death of the fish. The object of the following investigation was to determine whether extracts of any particular plant could be found which would act differentially, bringing about paralysis in certain varieties of fish while causing less harm to the others.

The importance of this problem arises from the fact that in the different tanks for stocking edible fish near the banks of the Ganges, undesirable species of fish get introduced during the rainy season from the overflowing river. One of these, the Bhetki (*Lates calcarifer*), is extremely voracious and cannibalistic in its habits, and subsists by destroying and eating other fishes preserved in the tanks. It is, therefore, an important question to be able to discover a particular plant, the extract from which, by its specific action on the *Bhetki*, might be effective in destroying the pest.

In a previous paper, published in the *Transactions of the Bose Institute*, vol. vii, investigations have been described on the action of the extract from the root of the Himalayan plant *Millettia pachycarpa* on different fishes.¹ It is there shown that this extract causes in different fishes an effective paralysis of the respiratory activity which brings about their death. A short summary of observations on the effect induced by the extract of *Millettia* on large numbers of fishes found in tanks is given below.

EFFECT OF EXTRACT OF *MILLETTIA PACHYCARPA*

Experiment 7. A dilute solution, 1 part of the root in 1000 parts of water, was employed in the following cases :

- i. *Tengara*.—A partial upset of the fish occurred in the course of 6 minutes. The respiratory activity became greatly depressed in the course of

¹ Bose, *Trans. Bose Institute*, vol. vii, p. 5.

- 15 minutes, and the fish died 18 minutes after the application with its opercula widely open.
- ii. *Rohit*.—A partial upset occurred in the course of 3 minutes ; this was followed by increasing and very pronounced difficulty of respiration. The fish collapsed and died after 20 minutes.
 - iii. *Katla*.—It began to struggle 3 minutes after the application of the extract ; an upset occurred in the course of 6 minutes, when the respiratory activity became spasmodic. The fish then struggled vigorously for a time, after which it became inert, and death occurred 18 minutes after the application of the extract.
 - iv. *Bhetki*.—As soon as the fish was placed in the extract it began to struggle, and a complete upset occurred 3 minutes after the application. The fish died in the course of 10 minutes.

In all the above typical cases the root extract in a solution as dilute as 1 in 1000 is found to be very virulent in its action, causing the death of different varieties of fish in a comparatively short time. *Millettia* cannot, therefore, be employed as a selective agent for destroying only the *Bhetki* fish.

There is, however, a belief entertained by some fishermen that the plant *Cajanus indicus* can be used for destruction of the *Bhetki*. It is said that when bundles of this plant are floated in the tank the active substance present in it becomes slowly dissolved in the water, causing paralysis and easy collection and removal of the particular pest. As soon as this has been accomplished, the floating bundles of *Cajanus* should be taken out of the water.

EFFECT OF EXTRACT OF *CAJANUS INDICUS*

The first investigations were undertaken to find which parts of the plant contained a comparatively large amount of the active principle. The results obtained showed that it was present to a considerable extent in fresh green leaves. Fully grown plants were also found to contain the active

principle in the bark of the stems. The following experiments were carried out with different fishes on the effect of extracts from green leaves in the moderately strong solution of 5 parts in a 1000. After carrying out this preliminary investigation, determination was made of the minimally effective dose.

Experiment 8. *Effect of extract from leaves of Cajanus, 5 : 1000.*—The first series of experiments were carried out with the above strength of solution on the following fishes: *Tengara, Rohit, Katla and Bhetki.*

- i. *Tengara.*—The fish became greatly disturbed under the action of this particular solution, there being a tendency towards an upset after an interval of 30 minutes. Death of the fish occurred 70 minutes after the application.
- ii. *Rohit.*—The fish began to be affected in the course of about 10 minutes, after which it frequently rose to the surface to take air through its mouth. After 30 minutes it lost its balance, and death occurred in the course of 65 minutes.
- iii. *Katla.*—In 12 minutes the fish exhibited the effect of the extract by rising frequently to the surface to inhale air. There was a tendency towards an upset 15 minutes after the application. The respiratory activity became irregular after 30 minutes, and death occurred in the course of 49 minutes.
- iv. *Bhetki.*—Under parallel conditions the *Bhetki* was found to react to the solution much earlier, death usually occurring in the course of about 25 minutes.

It will be seen from the experiments just described that the extract from the plant *Cajanus* is less virulent and more discriminative in its action than that of the root of *Millettia*; for while extract of *Millettia* as dilute as 1 : 1000 caused the death of different fishes in the course of 10 to 20 minutes, the five times stronger extract of *Cajanus* did not produce any fatal effect till the application had been continued for a very much longer period, which varied from 25 to 70

minutes. The virulence of the extract of *Cajanus* is, therefore, as already indicated, very much less than that of *Millettia*. Moreover, the selective action of *Cajanus* is distinctly indicated by the fact that the *Bhetki*, compared to the other fishes, is paralysed and killed in a much shorter time. It is worth noting in this connection that, while other fishes, removed from the solution after half an hour, became fully revived in ordinary water, the fish *Bhetki* could not be revived at all. These facts indicate that the extract obtained from *Cajanus* might prove effective in its selective action for the destruction of *Bhetki*.

EFFECT OF A MORE DILUTE EXTRACT OF *CAJANUS* *INDICUS*

Investigations were next carried out to find whether a still more dilute solution of *Cajanus* afforded a more clear indication of its selective action.

Experiment 9. *Effect of solution of Cajanus, 2 : 1000.*—The different edible fishes, *Tengara*, *Rohit* and *Katla*, were first subjected to the action of this dilute solution ; but none of them was adversely affected by it.

The effect of this particular dilute solution is, however, very marked in the case of *Bhetki* ; for under its action the fish exhibited considerable restlessness, and after only 2 minutes it became partially upset and unable to maintain its normal swimming position. After 6 minutes there was a total upset, the fish lying on its back upside down, gasping in its efforts to continue the respiratory activity. Every now and then there was a spasmodic struggle followed by a brief respite ; the fish, however, died after several such struggling efforts.

Experiment 10. *Effect of extract of Cajanus, 1 : 1000.*—Further experiments were carried out on *Bhetki* with a solution still more dilute, namely 1 : 1000. It has been shown (Expt. 9) that a dilution of 2 : 1000 had no effect on other fishes ; it is, therefore, obvious that they would be quite immune to the action of the still weaker solution of 1 : 1000. This very dilute solution was, however, found to be quite

effective on *Bhetki*, though slower in its action. The fish exhibited a partial upset in the course of 11 minutes, which was more pronounced after 25 minutes. Even in this condition the fish made repeated efforts now and then to come up and keep its head in the air above the surface of the water, but failed in its attempts, and subsequently expired.

The results, therefore, indicate that the extract from *Cajanus* is selective in its action in inducing paralysis and death of the pest.

An independent and still more striking method in demonstrating the action of extract of *Cajanus* on *Bhetki* is given below.

AUTOMATIC RECORD OF INDUCED VARIATION OF RESPIRATORY ACTIVITY

A series of automatic records of the variations of physiological activity induced in the fish by the extract was next obtained. The best means of securing this is to follow with the utmost accuracy the changes induced in the power of respiration of the fish so essentially necessary for the maintenance of its vital activities. The fish gets its necessary supply of oxygen from the air dissolved in the water, by means of its opercular pulsations which maintain the circulation of water round the gills. Any change in the amplitude or frequency of this opercular pulsation serves as a measure of variation of the vital activity induced by the action of the extract.

By means of the *Respirograph*, specially devised for the purpose, it has been possible to determine the effect of the extract in a continuous manner, from the initiation of the reaction to the final crisis culminating in death. The different phases in each normal pulsation, as well as the changes induced under the action of the extract, are thus recorded with unprecedented accuracy.

THE AUTOMATIC RESPIROGRAPH

For the purpose of obtaining records, the fish is laid on its side and, by means of a surgical bandage held on the support

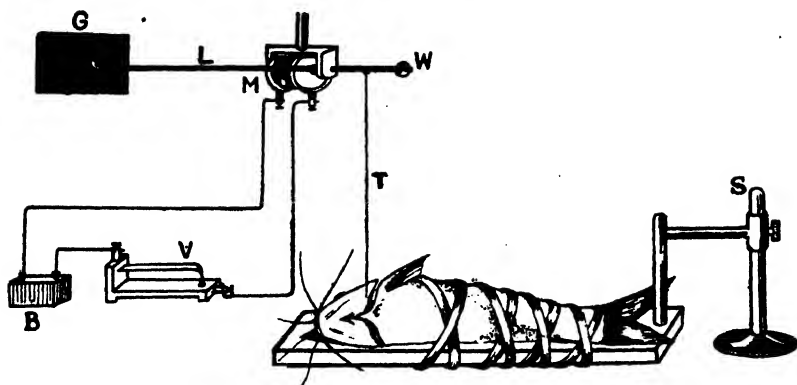


FIG. 2. Method of recording opercular pulsation by Resonant Respirograph.

Fish tied sideways by surgical bandage on wooden base; s, adjustable stand for keeping fish under water. Thin thread *t* passed through operculum, attached to short arm of writing lever, which is nearly balanced by counterpoise *w*. The steel writer of the *Resonant Recorder* is supported at centre of an electromagnet *m*, the magnetising coil of which is in circuit with storage cell *b* and vibrating reed *v* for periodic completion of electric circuit.

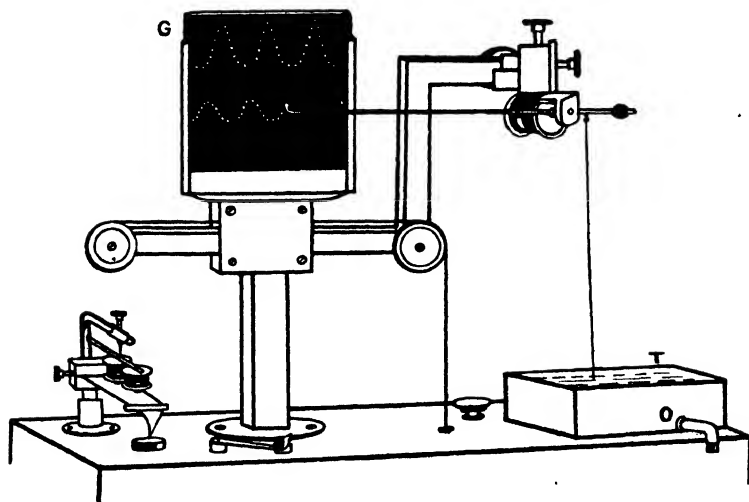


FIG. 3. Complete apparatus for recording the rate of respiratory activity of the fish.

t, trough for holding water or other chemical solution. (Fish not shown.) *o*, outlet pipe. The smoked-glass plate *G* can be raised 2.5 cm. at a time for successive records.

without undue pressure, kept in a quiescent condition (fig. 2). The fish thus secured is placed in a trough filled with water, and the normal record obtained as explained below; the particular solution is then introduced into the trough and the effect induced is recorded.

For obtaining accurate record of the respiratory activity and its induced variations, one of the opercula is attached, by means of a thin silk string, to one arm of the recording writer, which is maintained in resonant vibration by means of a reed which periodically completes an electric circuit; the resultant intermittent current of a definite frequency, flowing round a circular magnet, maintains the writing lever, constructed of a steel wire, in sympathetic vibration (fig. 3). The particular writer is tuned to vibrate 15 times in a second, and the interval between successive dots in the record is, therefore, $1/15$ part of a second. The record of normal pulsation, as already stated, is first taken, and the effect of the solution determined from subsequent series of records, of which those obtained at certain critical periods have been reproduced in the following figures.

Experiment 11. *Effect of extract of Cajanus, 2 : 1000.*—The series of records in fig. 4 exhibit the normal respiratory activity of the *Bhetki* and its induced variation under the action of the dilute extract. In the normal record N the up-curve represents the active movement of closure of the operculum, which is seen to be completed after 5 dot-intervals or in the course of $5/15$ second. The movement of opening or of relaxation was accomplished, on the other hand, in the course of $9/15$ second; in a complete pulsation the phase of relaxation is therefore slower than that of active contraction. A full pulsation is thus executed in $14/15$ second, its frequency being 64 per minute.

After the application of the extract, the character of each phase of the pulsation is seen to have undergone a definite variation, as indicated in the series (1) of the subsequent record. Though the frequency is found to have remained practically the same, yet the pulsations had become irregular and of an alternating character, the amplitudes of which are different. The contractile up-movement was not

much affected, but the movement of opening became somewhat more sluggish. After a longer period of application, the effect of the extract was still more pronounced, as seen in the series (2) of the record. The frequency of pulsation was also considerably slowed down, from the normal 64 to about 20 per minute. In each pulsation, moreover, the

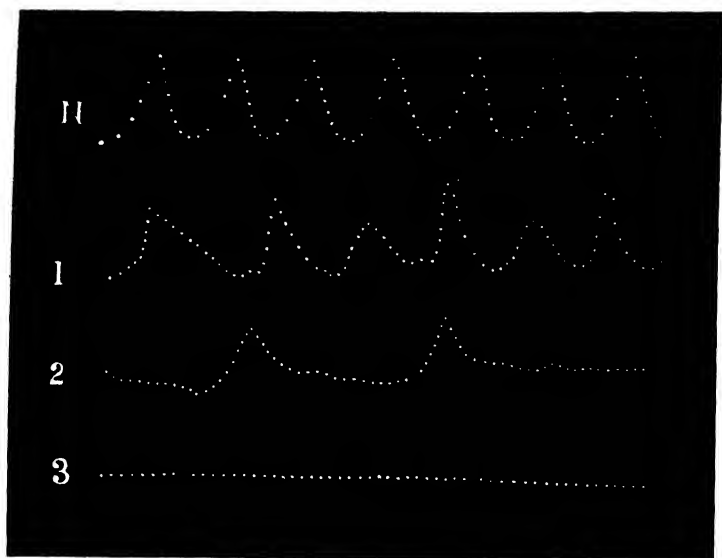


FIG. 4. Effect of dilute solution of *Cajanus*, 2 : 1000, on respiratory activity of *Bhetki*.

N is the normal record.

Series 1. Immediate effect.

Series 2. Effect of longer application.

Series 3. Arrest after application for 45 minutes.

(In this and in following records the up-curve represents active movements of closure, while the down-curve indicates movement of relaxation. Successive dots at intervals of $\frac{1}{15}$ second.)

period of opening or relaxation of the operculum, represented by the down-curve, became excessively prolonged, from the normal $9/15$ to more than $25/15$ second.

After a still longer period of application of the extract, i.e. for 45 minutes, the fish succumbed and the respiratory activity was completely abolished, as demonstrated by the horizontal line in series (3) of the record.

In the next experiment the solution was further diluted, and its effects were recorded on two different species of *Bhetki*, the first of which may be regarded as in an ordinary condition, while the second was extremely vigorous.

Experiment 12. *Effect of extract of Cajanus, 1 : 1000, on ordinary Bhetki.*—The normal record N in fig. 5 shows that the up-curve, representing closure movement, is completed in from $4/15$ to $5/15$ second, while the opposite move-

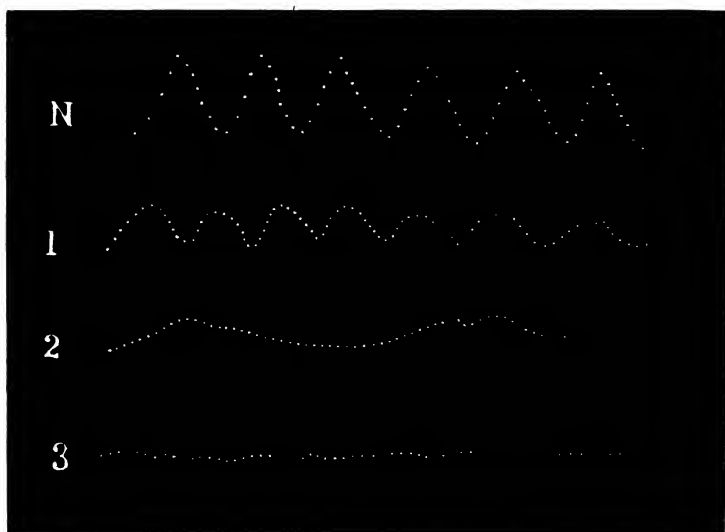


FIG. 5. Effect of dilute solution of *Cajanus*, 1 : 1000, on ordinary *Bhetki*.

N, the normal record ; the subsequent series 1, 2, 3 exhibit the effects of prolonged application of the solution.

ment of relaxation is executed in $7/15$ second, the frequency of pulsation being 75 per minute. The successive pulsations are seen to be very regular. But after the application of the extract the recorded pulses exhibited characteristic modifications as seen in series (1) of the subsequent record. The apex of the curve in the normal record was very sharp, but it became rounded under the action of the extract. The amplitude of the pulsation also became very greatly diminished.

After a longer duration of application of the extract, the pulsations became further reduced both in frequency and in amplitude, as seen in series (2) of the record, the frequency being reduced from the normal 75 to about 22 per minute. Later there was a complete abolition of respiratory activity,

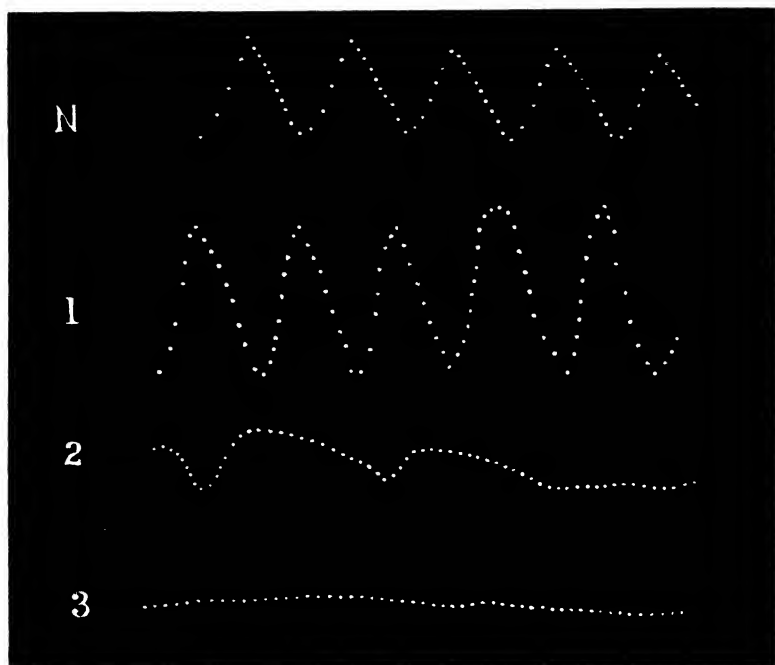


FIG. 6. Effect of dilute solution of *Cajanus*, 1 : 1000, on vigorous *Bhetki*.

N, the normal record ; the subsequent series 1, 2, 3 exhibit the effects of longer application of the solution.

Note preliminary stimulatory action, followed by depression and final arrest of respiration.

as demonstrated by the horizontal curve in series (3) of the record.

Experiment 13. *Effect of extract of Cajanus*, 1 : 1000, on a vigorous *Bhetki*.—The exceptional vigour of the specimen was noticeable from its very quick swimming movements. The amplitude of the opercular pulsation was relatively greater than that of the ordinary specimen, though the frequency was somewhat less. The record N in fig. 6 shows

that the frequency of pulsation was about 65 per minute. In each constituent pulsation the up-curve, representing closure movement, is completed, on an average, in $5/15$ second, while the opposite movement of relaxation is accomplished in $10/15$ second. In the ordinary specimen the application of the extract is immediately followed by a depression, as shown in fig. 5. In the present experiment with a vigorous specimen, the immediate effect of the extract was of a stimulatory character, which persisted for a certain length of time. This is shown by a considerable increase in the amplitude of pulsation, as demonstrated in series (1) of the record. The preliminary stimulatory reaction was, however, followed by a depression, shown by decrease in both the amplitude and frequency of the pulsation, as demonstrated in the series (2) of the record. After a still longer period, the respiratory activity was found to have been permanently abolished.

The results of investigations on the action of extracts of *Cajanus* show that, while a dilute solution induces no evil effect on the generality of fishes, it causes an arrest of the respiratory activity of the *Bhetki* culminating in its death. The plant *Cajanus* may, therefore, be regarded as a selective agent for the destruction of this particular fish.

SUMMARY

One of the objects of the present investigations was to find : Whether sound might exert any differential action on different fishes ; if not, whether water-disturbance has any such action. A further inquiry was undertaken in regard to the possibility of any differential action of certain vegetable extracts on different fishes.

The fishes examined were found not to respond in any way to the sound produced inside the water by an electric bell or to that of a high-pitched note.

In regard to the effect of water-disturbance produced by throwing clumps of earth into the water, there appears to be some indication, though not of a conclusive character, that fishes like *Katla* are less affected by this disturbance than the others.

In different tanks for stocking edible fish near the banks of the Ganges the fish *Bhetki* gets introduced from the over-flowing river during the rainy season. This fish, on account of its canibalistic habits, destroys other fishes preserved in the tanks. The problem to be solved is whether extract from certain plants, by its specific action on *Bhetki*, might be effective in destroying the particular fish.

The extract from the root of the plant *Millettia pachycarpa* is found very effective in the destruction of fishes in general. Being very virulent in its action, it cannot be employed as a selective agent for the destruction of *Bhetki*.

Investigations on the effect of extract from the plant *Cajanus indicus* showed that it was more discriminative in its action on different fishes. Two different methods of investigation were employed in the study of the effect of this plant extract, first that of eye-observation, and second that of automatic record.

The results of the first method of investigation showed that, while a solution obtained from *Cajanus* as dilute as 1 : 1000 did not produce any evil effect on the generality of fishes, it induced paralysis and death of the *Bhetki*. From the results of laboratory experiments it would appear that the plant *Cajanus* can, with due care, be utilised as a specific agent for the destruction of this fish.

The physiological activity of the fish is revealed by the automatic apparatus, which records the normal respiratory activity of the fish on which its life depends, as well as the changes induced in that activity by the action of the extract.

The respiration of the fish is maintained by the periodic closure and opening movements of its opercula. The *Resonant Respirograph* gives automatic records of the normal respiration and its induced changes, as also the time-relations of its phasic variations.

A very dilute solution of extract from *Cajanus* which has practically no effect on other fishes is fully effective on *Bhetki*, as demonstrated by the characteristic changes in its opercular pulsations, the movement of relaxation becomes more and more sluggish, and the fish is killed with its opercula widely open.

III.—RESPONSIVE MOVEMENTS OF LEAFLETS OF *AVERRHOA* UNDER STRONG LIGHT AND UNDER DARKNESS

BY

S. C. DAS, M.A., AND B. K. PALIT, B.Sc.

THE so-called 'sleep movement' of various plants has been the subject of detailed study by Darwin.¹ In regard to movements exhibited by the leaflet of *Averrhoa bilimbi*, Darwin found :

1. That the leaflets executed a series of down movements after 5 P.M., each of which consisted of a sudden fall followed by a slow rise through a shorter distance. The leaflet thus approached nearer the nocturnal down-position than it did after each previous fall. This nocturnal fall which occurs towards the evening might conceivably be ascribed to the effect of increasing darkness.
2. That strong sunlight induced an apparently similar effect ; for under the action of this excessively strong light the leaflets executed a series of descending steps precisely similar to those by which the nocturnal fall is effected. On shielding the leaflets from sunlight, they, under diffuse daylight, exhibited a slow and continuous recovery to the normal outspread position.

The apparent anomaly lies in the fact that under the two opposite conditions of darkness and of excessively strong light the leaflets exhibited

¹ Darwin, *The Power of Movements in Plants*, p. 330.

responsive movements which were apparently similar.

3. That the leaflets of *Averrhoa* exhibited, under conditions of favourable light and of warmth, induced pulsatory movements which were similar to the natural automatic movements of *Desmodium gyrans*.

For purposes of observation Darwin attached a long glass fibre to the leaflet, the pulvinus of which was at the centre of a graduated arc placed close behind the leaflet. The angles which the falling leaflet made with the vertical line were noted at frequent intervals, affording data for the construction of the curve of the responsive movements.

AUTOMATIC METHOD OF RECORD

The method of taking frequent angular measurements of the movements of the index is not only very laborious, but the results are also subject to personal errors inseparable from eye-observation. For obviating the above difficulties an automatic method was devised for the present investigation. The petiole bearing the leaflet of *Averrhoa* is held in a clamp, and a particular leaflet is attached to the short arm of the writing lever by a thin thread, the long arm of which gives a magnification of about 20 times.

The actual record is taken on a smoked glass plate (fig. 7) which moves laterally at a definite rate by means of clockwork. The plate itself is also made to oscillate to and fro by the same clockwork. The record, therefore, consists of a series of dots, the spacings between successive dots representing definite intervals of time. The responsive movements of the leaflet and their characteristics are thus recorded with great accuracy, and in a continuous manner. The time-relations of the responsive movements with their phasic variation can thus be determined from the dotted curve itself.

It is necessary to take certain precautions in order to obtain a prolonged record; for example, when after a series

of responsive falls, the leaflet is in a vertically downward position, its further movements can no longer be observed or recorded. In order to remove this drawback, the leaflet

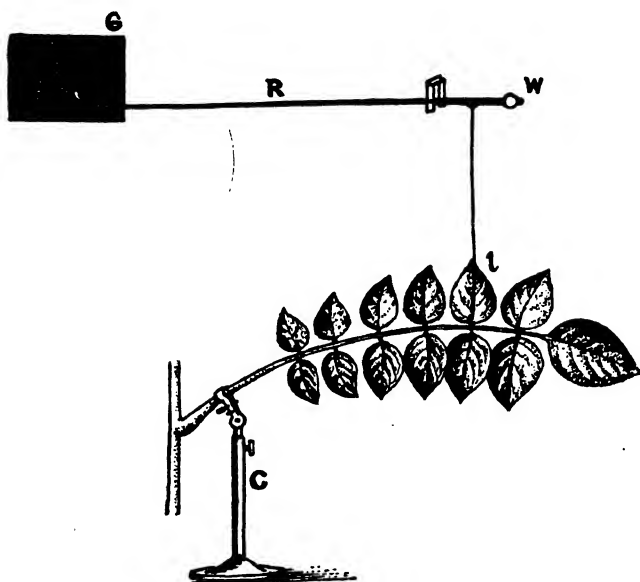


FIG. 7. Diagrammatic representation of the automatic method of record of the leaflet of *Averrhoa*.

The petiole of the leaf is held in clamp c ; the leaflet l is attached to the short arm of the writing lever, which exerts slight upward tension on the leaflet so as to retard its complete closure. The upward tension is due to the counterpoise w not exactly balancing the weight of the lever. The responsive movement of the leaflet is recorded by the long arm of the writing lever R. The smoked-glass plate G is made to move laterally at a definite rate by means of clockwork ; the plate itself is also made to oscillate to and fro.

attached to the recording lever is subjected to a moderate tension upwards, the object of which is to delay the complete closure of the leaflet.

THE EXPERIMENTAL ROOM

The experiments are carried out in a room which can either be completely darkened or diffusely illuminated by light from the sky. This can be effected by the opening or

closing of a wooden shutter. For the study of the effect of excessively strong light, since sunlight is not always available on account of the uncertainties of the weather, a strong parallel beam of light from a small arc lamp is employed. The strong light is thrown downwards on the leaflet by means of a small mirror suitably inclined. In order to cut off the heat rays, the parallel beam of light is made to pass through a thick stratum of alum solution.

The window of the room is provided with a thin muslin screen, which protects the leaflets from the disturbing action of currents of air when the shutter is open to admit light from the sky. The screen is sufficiently transparent fully to illuminate the room with diffuse light. Since the experimental room is large, its temperature was found to remain fairly constant for several hours at 30° C.

THE CHARACTERISTICS OF DIURNAL MOVEMENTS OF THE LEAFLET

It is the recurrent effects of light at daytime, and of darkness at night, impressed on the organism, that bring about the nyctitropic movements of the leaflets. The following experiments on the diurnal movements of *Averrhoa* were carried out, not only with the leaflets of *Averrhoa bilimbi*, but also with those of *Averrhoa carambola*. It is found that the period at which the evening fall commences is determined by the particular time when the afternoon light is undergoing a rapid diminution. In Calcutta, during summer months, the sun rises at about 5.30 A.M. and sets at 6.30 P.M. The rapidity of the decline of light in the afternoon is very marked at 5.30 P.M. and it is an important fact that only *after and never before* 5.30 P.M. is the evening fall of the leaflets initiated.

The commencement of the nyctitropic movement referred to above is somewhat modified by the age of the leaflets and the sensitivity of the specimen. In summer comparatively young leaflets begin to close after 5.30 P.M., but the older leaflets exhibit the commencement of their fall an hour later. The closure of the young leaflets is practically com-

plete by 6.45 P.M. ; but in the older leaflets it occurs about 2 hours later, *i.e.* by 8.30 P.M. They remain closed during the whole night and commence their movement of opening before dawn, between 4.30 and 5 A.M. This opening occurs even under the condition when the plant is kept in perfect darkness in a room with closed windows.

In regard to nyctitropic movements in general, observations were also carried out with other species of plants, the results being more or less similar. For example, in *Cassia alata* the evening fall of the leaflet commences after 5 P.M., and complete closure is effected by about 9 P.M. The leaflets remain closed till 4 or 5 A.M., after which they begin to open.

In the terminal leaflet of *Desmodium gyrans*, the closure movement commences after 5 P.M., and is completed by 8 P.M. The leaflet remains tightly closed throughout the night and begins to open and spread out early in the morning at about 5 A.M.

RECORD OF THE EVENING FALL OF THE LEAFLET OF *AVERRHOA BILIMBI*

Experiment 1. *The diurnal fall of the leaflet of Averrhoa bilimbi.*—The record is taken with the window open to admit daylight. The first part of the record obtained with this particular specimen (fig. 8) shows a horizontal line, which is indicative of the outspread position of the leaflet, this position being maintained till late in the afternoon. After 5.45 P.M., however, the leaflet exhibits a series of falls of a pulsatory nature, the characteristics of which are described below. The total number of these pulsations is 8, occurring in the course of 75 minutes. Out of the total number of such pulsations only 4 could be conveniently reproduced in the available space. In the present record the time-interval between successive dots is 2 seconds. The up-curve of the record represents the fall of the leaflet, while the down-curve exhibits a partial recovery.

Careful examination of the recorded curves shows that, taking the first complete pulsation as a typical example, the period of the fall is quick, while that of recovery is slow and

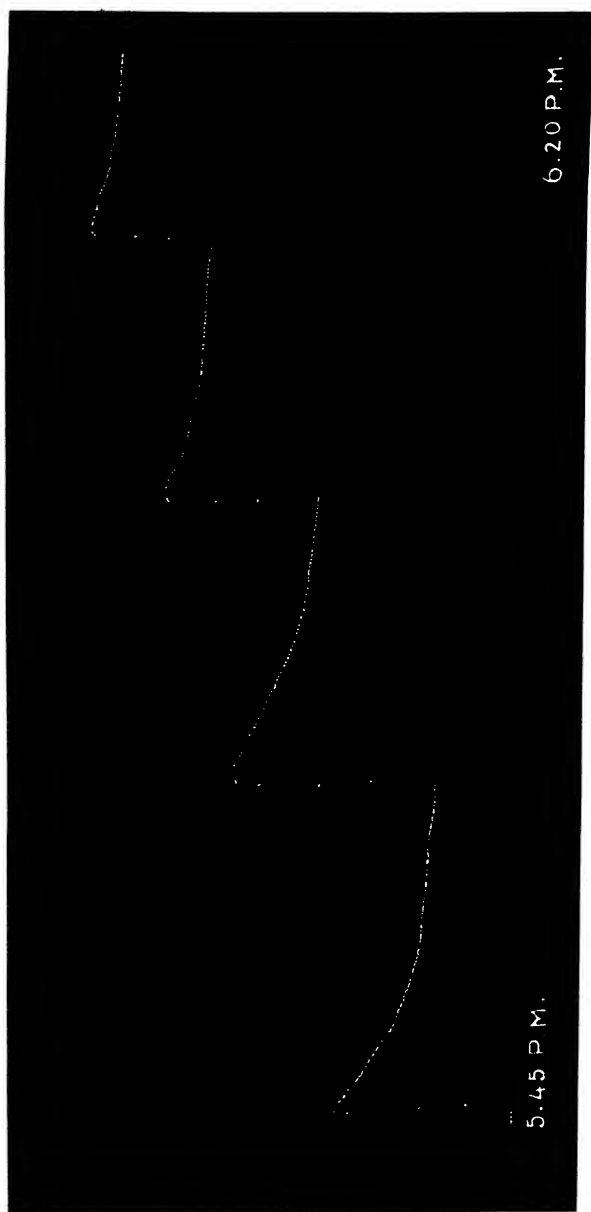


FIG. 8. Record of the evening closure of the leaflet of *Averrhoa bilimbi* which commenced at 5.45 P.M.
Successive dots at intervals of 2 seconds.

only partial. The duration of fall is represented by 4 spacings or $4 \times 2 = 8$ seconds. Owing to the slowness of recovery, the successive dots are nearly superimposed and therefore appear as almost continuous. The actual duration of the partial recovery is 14 minutes.

In the second pulsation the period of fall is 8 seconds, and that of recovery is about 12 minutes.

In the third pulsation the period of fall is 6 seconds, and that of recovery 11 minutes.

In the fourth pulsation the period of fall is 6 seconds, and that of recovery is 11 minutes. It is to be noted that the amplitude of the fall is undergoing a continuous diminution.

The characteristics of the four other pulsations which follow are similar to the above. The leaflet became completely closed by 7 P.M. after having executed the eighth pulsation. The total period occupied by the successive falls, as previously stated, is 75 minutes.

RECORD OF THE EVENING FALL OF THE LEAFLETS OF *AVERRHOA CARAMBOLA*

Experiment 2. *Diurnal fall of leaflet of Averrhoa carambola.*—In order to obtain the total number of pulsations in a single record, the speed of the lateral movement of the smoked-glass plate was reduced, and the oscillating plate was made to inscribe successive dots at intervals of 5 seconds. The evening fall of the leaflet was in this case initiated at 5.30 P.M., and the closure was completed by 6.15 P.M., after 6 complete pulsations, of which 5 are reproduced in fig. 9. The average period of each pulsation was 7.5 minutes.

In the first pulsation the period of fall was 7×5 or 35 seconds, and the period of recovery was 11 minutes.

In the second pulsation the amplitude of the fall was reduced; the period of fall was 30 seconds and a partial recovery was effected in the course of 10 minutes.

In the third pulsation the amplitude was further reduced; the period of fall was 25 seconds, there being a partial recovery in the course of 8 minutes.

In the fourth pulsation the period of fall was 15 seconds,

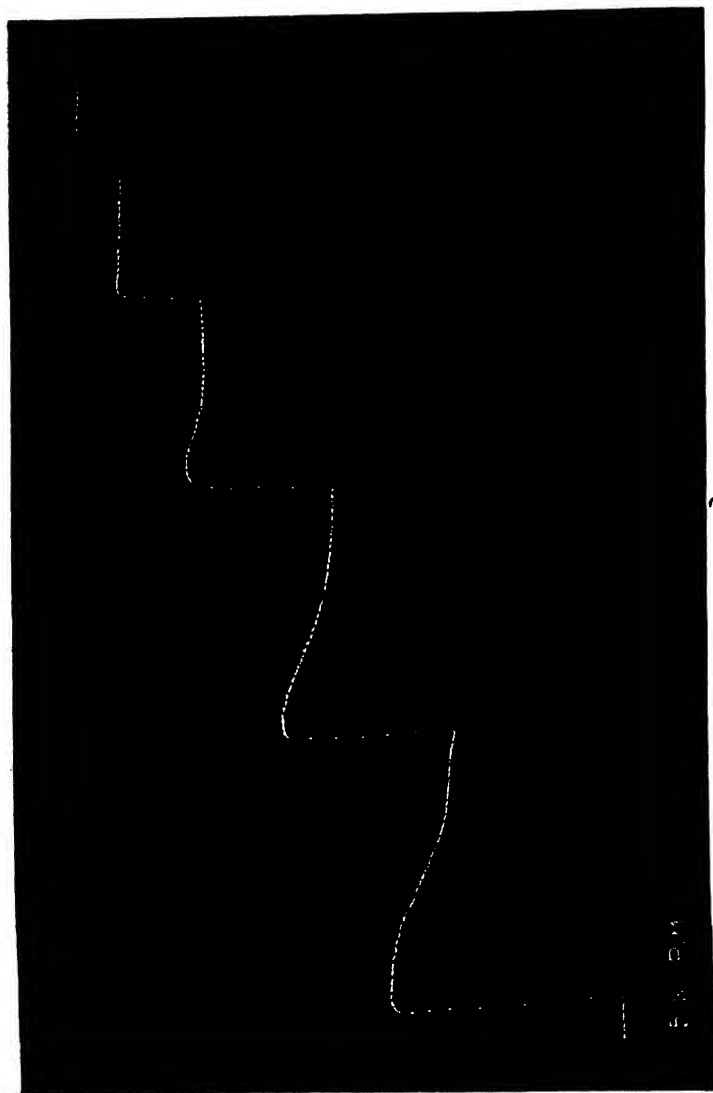


Fig. 9. Record of evening closure of the leaflet of *Averrhoa carambola*.
Successive dots at intervals of 5 seconds.

and that of recovery was 5.5 minutes. The amplitude of pulsation had, as before, undergone a decline.

In the fifth pulsation the period of fall was 10 seconds, while partial recovery occurred in the course of 4·5 minutes.

In the sixth pulsation the duration of fall was 5 seconds, and the partial recovery was effected in the course of 4 minutes. After this the leaflet became completely closed.

The next series of observations were undertaken on the effect of strong light on the leaflets of *Averrhoa*.

EFFECT OF STRONG LIGHT ON *AVERRHOA BILIMBI*

Experiment 3. *Record on a slow-moving plate*.—This experiment was carried out in a room illuminated by diffuse daylight. The leaflet was in the normal outspread position which remained perfectly steady; the record was therefore a horizontal line, the successive dots of which were at intervals of 5 seconds. On application of strong light from the arc lamp, the pulsatory responsive fall of the leaflet was initiated in the course of about 15 seconds.

It should be stated in this connection that with a much higher magnification a slight and brief erectile movement of the leaflet can be noticed, due to the contraction of the directly stimulated upper half of the pulvinule. This response is subsequently reversed to one of fall in consequence of transverse conduction of excitation to the lower half of the organ, which is relatively the more excitable. As already stated, the brief preliminary erectile movement is not shown in the record under moderate magnification.

Examination of the responsive series of falls under strong light shows that the first responsive fall is completed in the course of 2 spacings or 10 seconds (fig. 10). After a partial recovery there occur the second and other pulsations in sequence. The amplitudes of the successive movements of fall become less and less. As a result of incomplete recoveries in each pulsation, the leaflet falls in stages to its utmost vertical down-position.

The effect of strong light in the present case is exhibited by 5 pulsations, occurring in the course of 100 seconds, the average period of a single pulsation being 20 seconds. On the removal of strong light and restoration of diffuse day-

light, the leaflet exhibits a slow recovery which is nearly complete in the course of about twenty minutes.

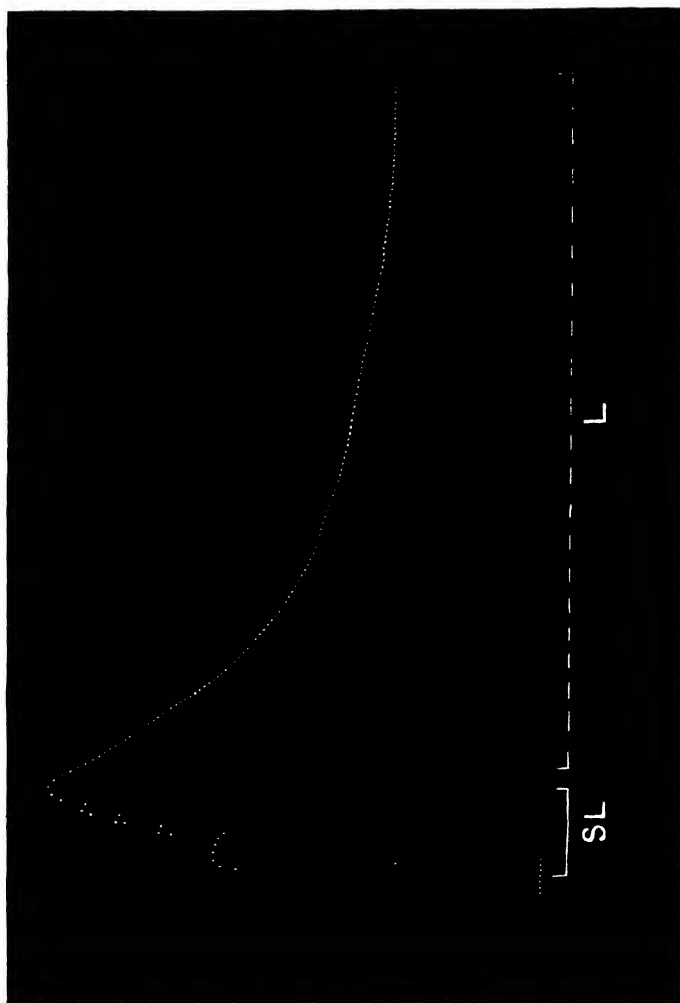


FIG. 10. Pulsatory falls of leaflet of *Averrhoa bilimbi* under strong light SL; recovery under diffuse light L.
Successive dots at intervals of 5 seconds.

Experiment 4. *Record on a fast-moving plate.*—It is difficult to determine very accurately the characteristics of the successive pulsations when the plate for obtaining record is moving at a slow rate. This difficulty is overcome in an

experiment with a fresh specimen of *Averrhoa bilimbi*, by taking the record on a fast-moving plate, the successive dots being made at shorter intervals of 2 seconds. The pulse-record thus became sufficiently spread out for the accurate determination of the time-relations. The record (fig. 11) shows that the pulsatory fall of the particular leaflet is initiated in the course of 10 seconds after application of

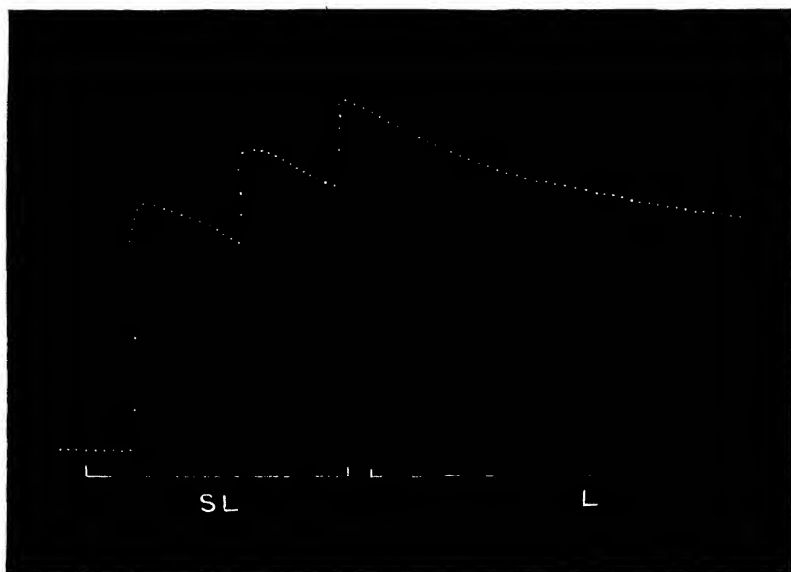


FIG. 11. Record of the effect of strong light SL on the leaflet of *Averrhoa bilimbi*, taken on a faster moving plate; recovery under diffuse light L.

Successive dots at intervals of 2 seconds.

the strong light. In this, as in the previous record, the amplitudes of successive falls become less and less. In the first pulsation the period of fall is about 12 seconds and that of partial recovery 24 seconds. In the second pulsation the fall occurs in the course of 8 seconds, and the partial recovery is effected after 22 seconds. In the third pulsation the period of fall is 8 seconds, and that of partial recovery about the same as before.

It may be stated that when the amplitude of an individual

pulsation is exceptionally large, then the complete closure of the leaflet occurs after a few pulsations. In the present instance there are 3 pulsations completed in the course of 97 seconds, the average period of a single pulsation being about 33 seconds.

EFFECT OF STRONG LIGHT ON *AVERRHOA CARAMBOLA*

Experiment 5. *Record on a slow-moving plate.*—The leaflet of *Averrhoa carambola* exhibited effects essentially

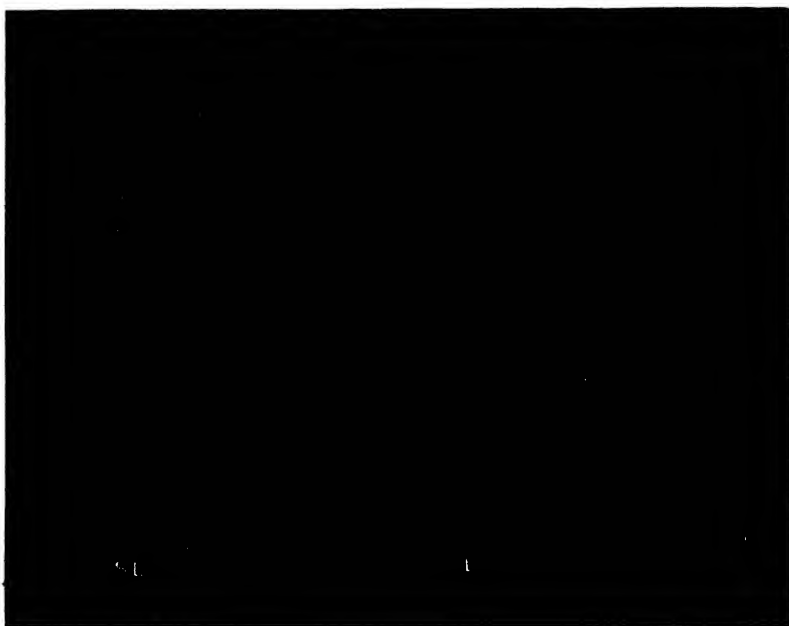


FIG. 12. Record of the effect of strong light SL on the leaflet of *Averrhoa carambola*; recovery under diffuse light L.

Successive dots at intervals of 5 seconds.

similar to those of the leaflet of *Averrhoa bilimbi*. The only noticeable difference is in the relative sluggishness of the response of *Averrhoa carambola*, which is comparatively less sensitive to the action of light.

After application of the strong light a responsive fall

was initiated after 25 seconds, followed by others. The first of the series of falls was completed after 20 seconds, after which a partial recovery occurred in about the same length of time. Somewhat similar results characterised the second and third pulsations. The amplitudes of successive pulsations underwent the usual decline, the leaflet falling vertically down to its utmost after the third pulsation. The average period of a single pulsation of the series of three was found to be about 40 seconds. On stoppage of the strong light, the leaflet, under diffuse illumination from the sky, exhibited a slow recovery, which was nearly complete in the course of about 25 minutes (fig. 12).

After this the leaflet retained its fully outspread position in the diffused light from the sky till the commencement of the evening fall several hours later.

Though strong light induced a series of falls, similar to those which brought the leaflet to its nocturnal position on account of increasing darkness in the evening, yet certain differences are noticeable in the characteristics of the two movements. This will be understood from the following summaries of results obtained from previous experiments.

Characteristics of the Nocturnal Fall

Average period of a single complete pulsation in :

(a) <i>A. bilimbi</i> (Expt. 1)	.	.	.	9	minutes
(b) <i>A. carambola</i> (Expt. 2)	.	.	.	7.5	„

Mean 8.25 minutes

The nocturnal fall commenced only *after* 5.30 P.M. and *never before* that hour.

Characteristics of Fall under Strong Light

Average period of a single complete pulsation in :

(a) <i>A. bilimbi</i> (Expt. 3)	.	.	.	20	seconds
(b) <i>A.</i> „ (Expt. 4)	.	.	.	33	„
(c) <i>A. carambola</i> (Expt. 5)	.	.	.	40	„

Mean 31 seconds

It will be noted that while the mean period of a single pulsation of nocturnal fall of the leaflet of *Averrhoa* is 8.25 minutes, that under strong light is only 31 seconds. It should be borne in mind in this connection, that the quickness of fall under strong light depends on the intensity of the incident light.

A further discriminating test between the two movements is the fact already referred to, that the leaflet under diffuse light maintained its outspread position for hours till the nocturnal fall was initiated after 5.30 P.M.

EFFECT OF STRONG LIGHT FOLLOWED BY COMPLETE DARKNESS

The experiments that have already been described relate to the effect of strong light being followed by diffuse light from the sky. Strong photic stimulation has been shown to give rise to a multiple series of responsive falls of the leaflet, the recovery into outspread position being exhibited on restoration of daylight. The question arises: what would happen if strong light was followed not by diffuse light from the sky, but by complete darkness?

Experiment 6. *Effect of strong light followed by darkness.*—The following may be regarded as a typical result obtained with the leaflet of *Averrhoa carambola*. The leaflet was in the normal outspread position in daylight. On the application of strong light from the arc lamp, a series of pulsatory falls were produced till the leaflet became fully closed into its vertical down-position, after having executed three complete pulsations. The successive dots in the record were at intervals of 5 seconds. The amplitudes of the successive pulsations exhibited, as before, a continuous diminution. The average period of a single complete pulsation in this case was approximately 48 seconds (fig. 13).

The strong light was next cut off, and the room fully darkened by closing the shutters of the window. The leaflet exhibited in this darkness a slow recovery from the effect of strong light, this being nearly complete in the course of about 20 minutes. In the previous experiments the *average period*

of recovery in diffuse light of the sky is about 22 minutes. There is thus no apparent difference between the recovery under diffuse light or under complete darkness. Is there then no difference in the after-effect under the two above conditions? The following investigation was undertaken to solve this problem, by continuing the automatic record of

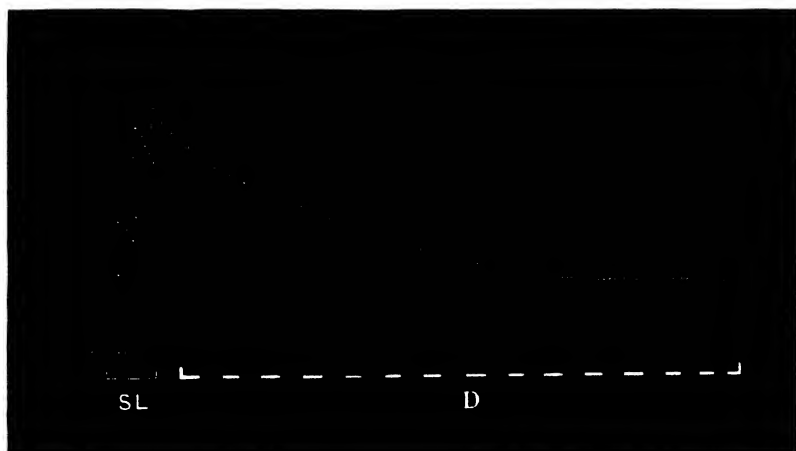


FIG. 13. Record of the effect of strong light SL on the leaflet of *Averrhoa carambola*; subsequent recovery in darkness D. Successive dots at intervals of 5 seconds.

the movement of the leaflet in complete darkness for a very long time, even after the recovery has apparently been effected.

EFFECT OF STRONG LIGHT FOLLOWED BY PROLONGED DARKNESS

Experiment 7. *Response of Averrhoa bilimbi*.—The experiment was commenced at 10 A.M., when under diffuse light from the sky the leaflets were in the normal outspread position. On application of strong light from the arc lamp the leaflet exhibited the normal series of pulsatory falls, there being 6 pulsations in the course of 3 minutes. The average period of a complete pulsation was therefore 30 seconds. The room was next completely darkened and the leaflet recovered its outspread position in the course of

about 25 minutes. After this recovery the record was continued in complete darkness for additional periods which, in different cases, varied from 20 to 30 minutes.

During this prolonged period of darkness, the leaflet now exhibited very striking additional responsive reactions of much significance, which showed certain characteristics different from those of the nyctitropic fall. This particular

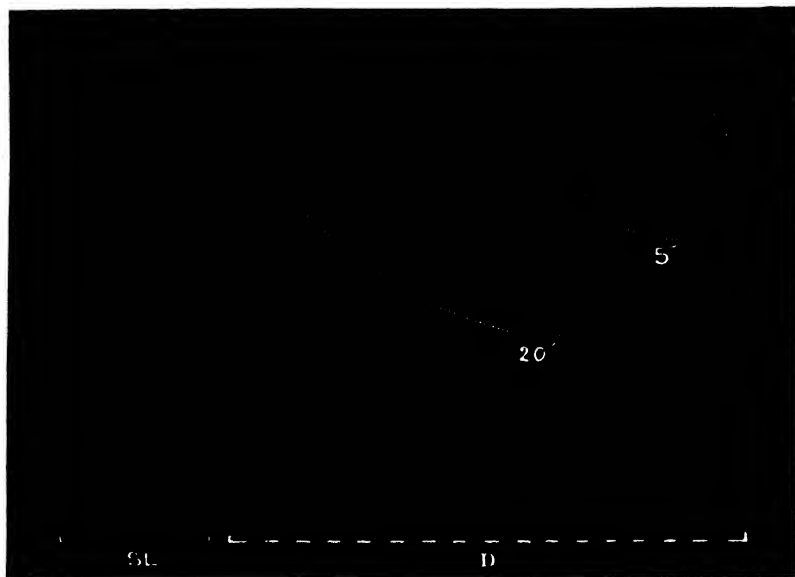


FIG. 14. Record of the effect of strong light SL on the leaflet of *Avertrhoa bilimbi*. Subsequent responses in prolonged darkness D, of which only two are shown.

Successive dots at intervals of 5 seconds.

effect may conveniently be distinguished as *Response to Darkness*.

Returning to the above effect of prolonged darkness in *Avertrhoa bilimbi* the specific response consisted of a series of pulsatory falls of the leaflet, of which only two are reproduced. The first of these falls was initiated 20 minutes after the apparent recovery in darkness (indicated in the record as 20'), and the second fall occurred 5 minutes after recovery from the first (fig. 14). The amplitude of each of

these pulsatory falls in darkness is so large that the leaflet was soon brought to the vertical down-position.

A special characteristic of the response to darkness is the extraordinarily great rapidity with which the fall is effected. Careful examination of the record shows that this

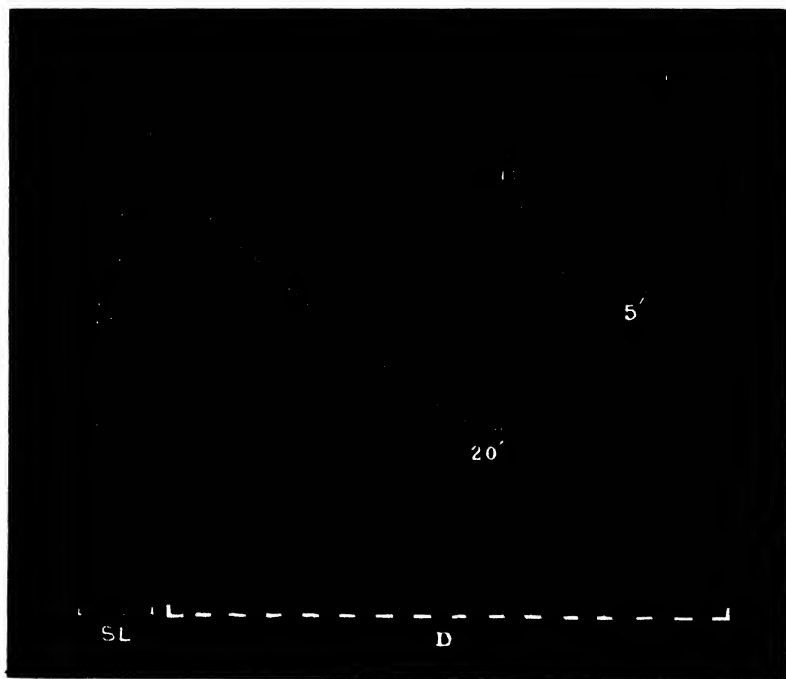


FIG. 15. Record of the effect of strong light SL on the leaflet of *Averrhoa carambola*. Subsequent responses in prolonged darkness D.

Successive dots at intervals of 5 seconds.

fall is practically complete after a single jump which occupied only 5 seconds; the responsive fall and subsequent partial recovery occur in the course of 3 minutes; the second pulsation, as previously stated, is initiated after a further interval of 5 minutes, the fall being as rapid as in the first case.

Experiment 8. *Response of Averrhoa carambola*.—The

experiment was commenced at 9 A.M. and the results are found to be essentially similar to those of *Averrhoa bilimbi*. The leaflet in diffuse light was in its normal outspread position; application of strong light induced a series of three pulsatory falls, each of which was completed in about 15 seconds; the average period of a single pulsation, consisting of the fall and partial recovery, was about 25 seconds.

The time for recovery into outspread position of the leaflet in complete darkness was 25 minutes. Prolonged darkness for another 20 minutes gave rise to a further series of abrupt falls of the leaflet, only two of which are reproduced. The characteristic of the response of *Averrhoa carambola* to prolonged darkness is thus similar to that of *Averrhoa bilimbi*. In each case the fall of the leaflet is very abrupt, being completed in the course of 5 seconds. Moreover, the amplitude of fall in response to darkness is so great, that the leaflet was brought to its utmost down-position as under intense light (fig. 15).

CHARACTERISTIC DIFFERENCES IN THE THREE TYPES OF PULSATORY FALLS OF THE LEAFLET

In making a survey of the three types of the apparently similar pulsatory falls of the leaflet, these may be classified as follows:

1. The fall occurring in the evening when light is undergoing a rapid diminution.
2. The fall under the action of very strong light.
3. The fall under the action of prolonged darkness.

In spite of the apparent similarity between the three types of reactions described above there are, however, specific differences by which they may be distinguished from each other. This will be understood from the following characteristics of each type.

1. *The evening fall*.—This occurs only when light is undergoing a rapid diminution at or after 5.30 P.M., and never before that time. The amplitude of the

movement of fall is only moderate, and each constituent pulsation is completed in about 8 minutes.

2. *The responsive movements under strong light followed by diffuse light.*—Each fall under strong light, though apparently similar to that of nyctitropic fall, differs nevertheless from the latter by the fact : (a) that it can be initiated at any hour of the day ; (b) that the average period of each constituent pulsation is only about 32 seconds ; and (c) that the recovery in diffuse daylight occurs in the course of about 20 minutes or so. After this the leaflets remain outspread for many hours till the commencement of the evening fall.
3. *The responsive movements under strong light followed by prolonged darkness.*—The sequence of these responses is as follows : (i) a series of quick falls under strong light ; (ii) an apparent recovery in darkness (in the course of about 20 minutes) as under diffuse light ; and (iii) additional pulsatory falls of the outspread leaflet to the utmost down-position under the action of prolonged darkness. In regard to these responses to prolonged darkness the characteristics are : (a) the very large amplitude of each fall ; (b) the extraordinary rapidity with which the fall is effected ; and (c) its occurrence at any time of the day, and not necessarily in the evening.

The above results prove that the response to darkness is specifically different from that induced under the action of strong light, or from that of the nyctitropic fall induced in the evening.

Hitherto the response to darkness was obtained with a leaflet that had been previously subjected to the action of strong light. The question arises whether previous exposure to strong light (as in Experiments 6, 7, and 8) is at all necessary for the exhibition of this particular form of response. The following investigations were undertaken in answer to this inquiry.

SPECIFIC RESPONSE OF LEAFLETS OF *AVERRHOA* TO DARKNESS WITHOUT PREVIOUS EXPOSURE TO STRONG LIGHT

In the following experiments the plant after the previous night's darkness is kept under feeble light in the experimental room and not exposed to bright daylight. The following experiment was commenced at about 8 A.M.

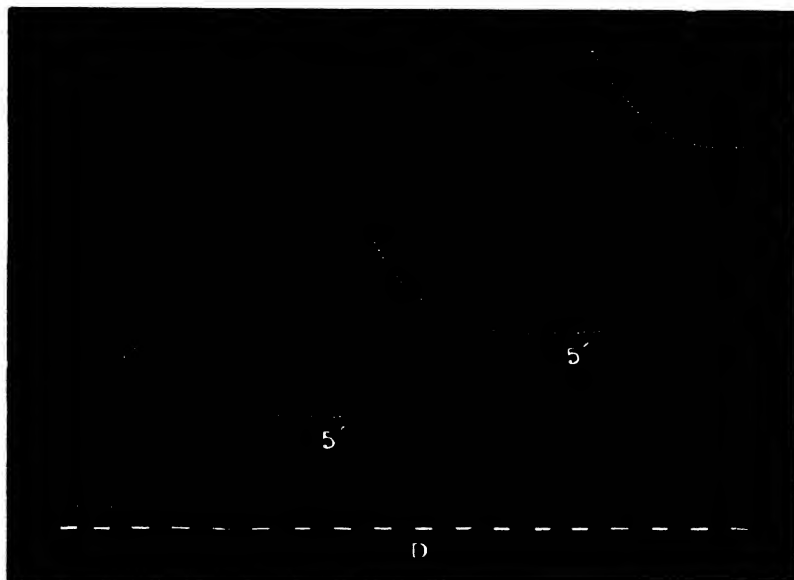


FIG. 16. Response to darkness (*Averrhoa bilimbi*).
Successive dots at intervals of 5 seconds.

Experiment 9. *Response of leaflet of Averrhoa bilimbi to darkness.*—One of the conditions for obtaining satisfactory results is the choice of a sufficiently sensitive young leaflet. Special care has also to be taken so that the leaflet selected for the experiment should be in a quiescent condition. After taking the horizontal record of the leaflet in the normal outspread position under feeble light, the room was completely darkened. Under the action of this darkness a series of responsive falls were initiated in the course of 15 minutes. These responsive falls of the leaflet, which were

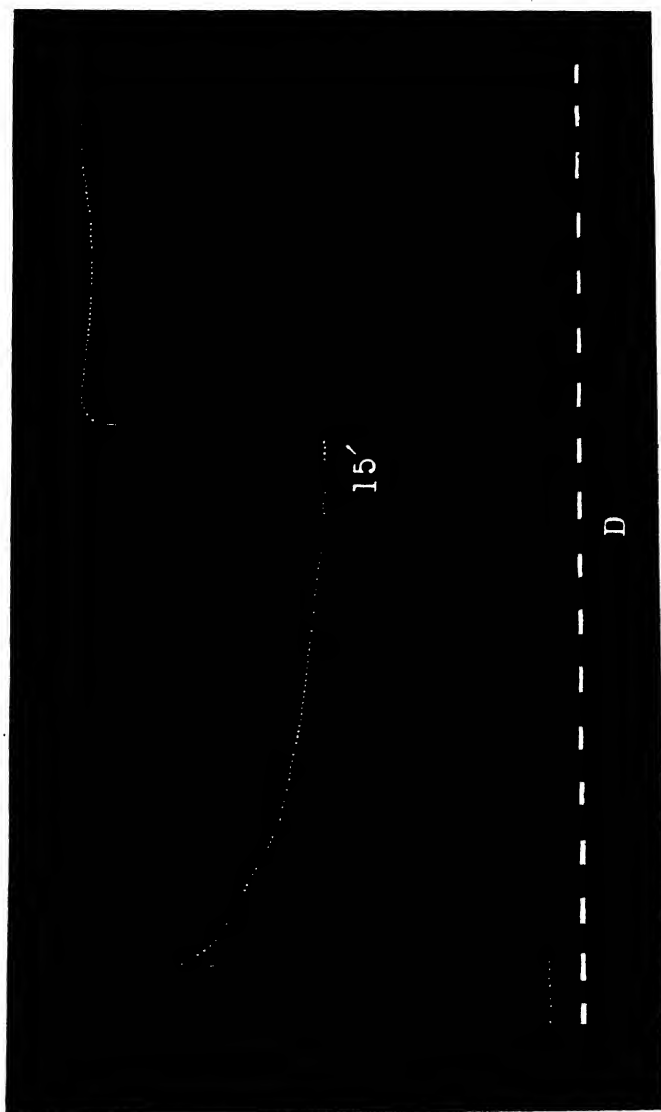


FIG. 17. Response to darkness (*Averrhoa carambola*).
Successive dots at intervals of 5 seconds.

very abrupt and of large amplitude, were completed in a time as short as 5 seconds. The first fall and partial recovery were effected in the course of 3 minutes; the subsequent

responsive falls of the series occurred each after a further interval of 5 minutes. The amplitudes of the falls were so great that the leaflet fell to its utmost down-position in a very short time (fig. 16).

Experiment 10. *Response of the leaflet of Averrhoa carambola to darkness*.—The leaflet of this species of plant is less sensitive than that of *Averrhoa bilimbi*, and it is probably on this account that it required the longer subjection to darkness of an hour to initiate the characteristic series of responsive falls. The rapidity of the fall was, as in the other cases, very great, being completed in about 5 seconds; on account of the sluggishness of the leaflet a partial recovery was produced in the course of the longer period of 8 minutes. It was after a further period of 15 minutes subsequent to the partial recovery that the second pulsatory fall occurred (fig. 17).

There is, therefore, a specific response to darkness which is different from that caused either by strong light or by increasing darkness in the evening. To make this point quite clear the important difference may be stated once more. The response to strong light and *subsequent diffuse illumination* is a series of responsive falls followed by recovery. The response to strong light and *subsequent prolonged darkness* is not merely a series of falls followed by recovery as in the last case, but there are additional responses specifically due to darkness, the characteristics of which are the very large amplitudes of the fall as well as their extraordinarily great rapidity. The response to darkness can also be obtained without previous exposure to strong light. In regard to the nyctitropic fall of the leaflet, this occurs only after 5.30 P.M., while the fall of the leaflet under excessively strong light or under prolonged darkness can be produced at any time of the day.

AUTONOMOUS RESPONSE OF *AVERRHOA* AND ITS CONTINUITY WITH MULTIPLE RESPONSE

It has been shown elsewhere that while in many plants a single moderate stimulus evokes a single response, a strong

stimulus gives rise to a recurrent series of responses.¹ The energy of the incident stimulus thus appears to become latent for the time being, finding expression in subsequent series of multiple or recurrent responses. Under natural conditions the plant is exposed to the action of various stimuli supplied by its environment. It is exposed to heat, to the action of light, to the mechanical stimulus of air-currents, and to the action of various chemical stimuli present in it or absorbed by it. From the joint action of these external sources of stimulation, the energy stored up by the plant becomes sufficiently great to cause an excitatory overflow, exhibited in movements which appear to be automatic. The potentiality of such automatic movements, often described as autonomous, is in reality derived from external stimuli, the energy of which has become stored up in the organism.

MULTIPLE RESPONSE

Experiment II.—*Multiple responses of leaflet of Bio-phytum*.—It is easy to induce multiple responses of the leaflet of this plant under the action of strong light. For the establishment of the universality of the phenomenon, a different mode of stimulation was, however, employed in the present experiment, in which strong stimulus was applied from an electro-thermic stimulator, which consisted of a V-shaped loop of wire, heated short of incandescence by the passage of an electric current. The intensity of incident heat rays from the V-shaped loop can either be maintained constant or suitably varied by approach or recession of the radiating loop to and from the leaflet. A series of thermal shocks can also be applied in rapid succession by means of a metronome, which closes the electric circuit.

In fig. 18 is seen a series of multiple responses produced under the strong electro-thermic stimulation *Th*. The successive dots in the record are at intervals of 2 seconds. There are four pulsatory falls, the average period of which

¹ Bose, *Plant Response* (1906) ; *Comparative Electro-Physiology* (1907).

is 4 seconds. The partial recovery was effected in different cases in the course of 12 to 30 seconds, the average period of a single complete pulsation being about 21 seconds. The series of multiple responses, due to the single strong stimulus, was on its cessation followed by a gradual recovery.

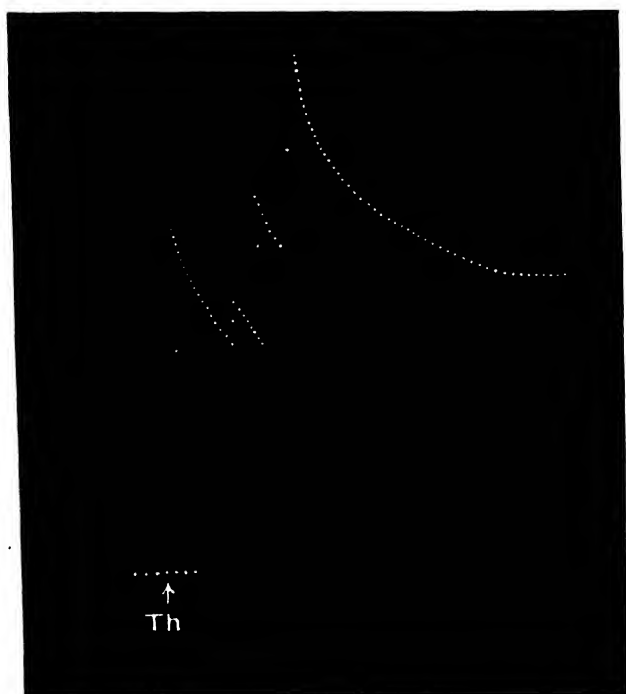


FIG. 18. Multiple responses of leaflet of *Biophytum sensitivum* under electro-thermic stimulation *Th*.

Successive dots at intervals of 2 seconds.

Experiment 12. *Multiple responses of leaflet of Averrhoa bilimbi*.—The multiple response of the plant under strong photic stimulation has already been referred to. In the present experiment the application of electro-thermic stimulation *Th* gave rise to a series of 4 multiple responses, the first of which was initiated after a latent period of 8 seconds (fig. 19).

Experiment 13. *Multiple responses of leaflet of Averrhoa carambola*.—Under electro-thermic stimulation *Th*, multiple

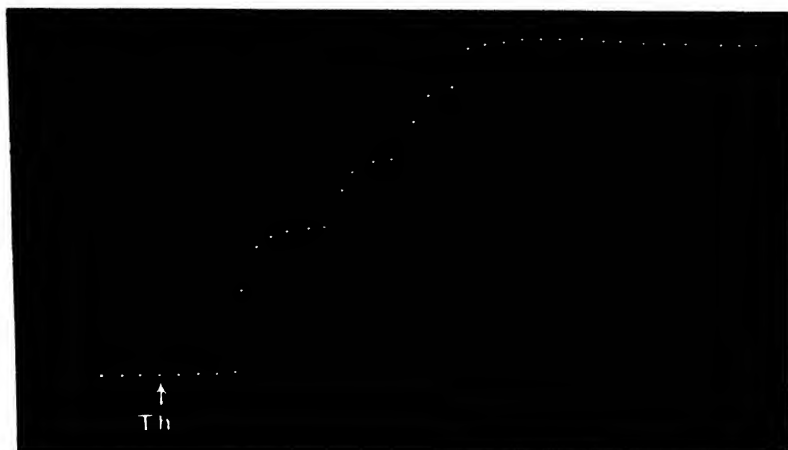


FIG. 19. Multiple responses of the leaflet of *Averrhoa bilimbi* under electro-thermic stimulation *Th*.
Successive dots at intervals of 2 seconds.



FIG. 20. Effect of electro-thermic stimulation *Th* on the leaflet of *Averrhoa carambola*.
Successive dots at intervals of 2 seconds.

response of the leaflet was initiated after a latent period of 6 seconds, there being three pulsatory falls (fig. 20).

AUTONOMOUS RESPONSE

Experiment 14. *Autonomous pulsation of the leaflet of Aerrhoa bilimbi*.—Under favourable conditions of light and

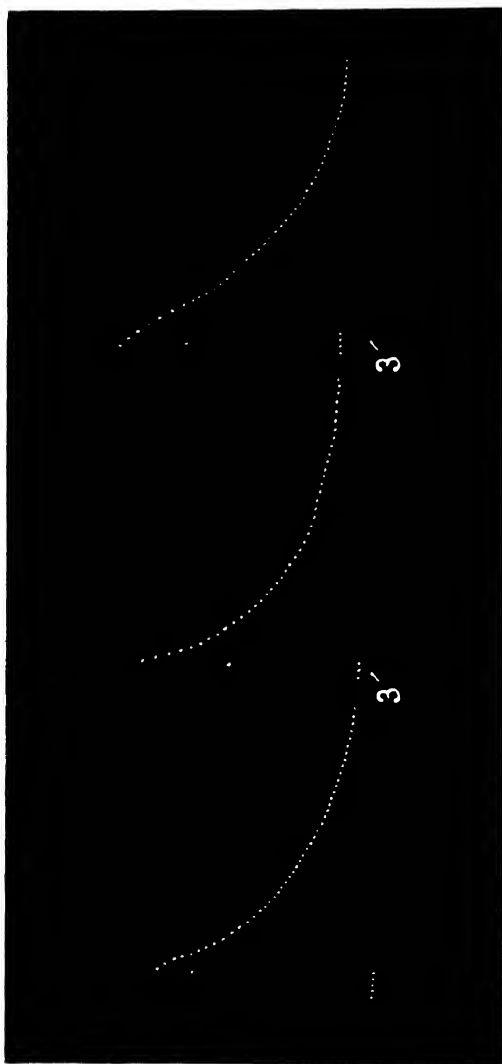


FIG. 21. Uniform autonomous pulsations of the leaflet of *Aerrhoa bilimbi*.
Successive dots at intervals of 5 seconds.

warmth, the sensitive young leaflet of this plant exhibits a uniform series of autonomous pulsations. In the record of

autonomous pulsations of the leaflet of *Averrhoa bilimbi* which is reproduced in fig. 21, the contractile fall is very rapid, while the recovery is comparatively slow. Detailed examination of the pulsations shows a quick fall which is completed in 10 seconds, the period of recovery of the leaflet to its outspread position being 4.5 minutes. After a further interval of 3 minutes, represented by gaps in the record, the second and subsequent pulsations were initiated. The record shows only three such pulsations which are characteristic of a long series; the three pulsations referred to were completed in the course of 22.5 minutes, the average period of a single pulsation being 7.5 minutes.

The characteristics of these pulsations are essentially similar to the automatic pulsations of the leaflet of *Desmodium gyrans*. In both the period of the fall of the leaflet is relatively rapid while that of erectile recovery is slow. In the leaflet of *Desmodium gyrans* the average period of a single pulsation under normal conditions is found to be 3 minutes, that of *Averrhoa*, as already stated, being 7.5 minutes. The autonomous activity of the *Averrhoa* leaflet may, therefore, be regarded as 2.5 times slower than that of *Desmodium*. In other respects also the pulsatory activity of the two different leaflets is modified alike under variation of temperature and under the action of narcotics.

SUMMARY

The leaflets of *Averrhoa bilimbi* as well as those of *Averrhoa carambola* exhibit nyctitropic fall in the evening. This occurs in Calcutta during the summer months after 5.30 P.M., and never before that time. The nyctitropic movement consists of a series of pulsatory falls, each of which is followed by a slow rise through a shorter distance, the final result being the complete closure of the leaflets.

For accurate determination of the responsive movements of the leaflets, an automatic method has been devised by which the pulsatory movements and their characteristics are recorded with great accuracy and in a continuous manner. The time-relations of the responsive movements

in their phasic variation can thus be determined from the recorded curve itself.

Under the action of excessively strong light, the leaflets of *Averrhoa* execute a series of descending steps apparently similar to those of the nocturnal fall. There is, however, a distinct difference between the two cases. The nocturnal series of falls occur only after 5.30 P.M., whereas the responsive falls under excessively strong light can take place at any time of the day. Moreover, while the average period of a single complete pulsation during the nocturnal fall is about 8.25 minutes, the average period of a single pulsation under strong light is 31 seconds only.

After a series of pulsatory falls under excessively strong light, the leaflets of *Averrhoa* exhibit, on the cessation of strong light, a recovery to the normal outspread position in the course of about 20 minutes or so. This recovery occurs equally well when the strong light is followed by diffuse light from the sky or by darkness.

Prolonged darkness is found to give rise to additional pulsatory falls which may be regarded as specific response to darkness. The characteristics of these responses are : (a) the very large amplitude of each fall ; and (b) the extraordinary rapidity with which the movement of fall is effected.

The leaflets of *Averrhoa* exhibit multiple responses to strong stimulus. This is true not only of the photic but also of other modes of stimulation, such as the electro-thermic.

It has further been shown, that there is a continuity between the multiple and the autonomous response. The potentiality of autonomous movements is in reality derived from external stimuli, the energy of which has become stored up in the organism.

Under favourable conditions of light and warmth, the leaflet of *Averrhoa* exhibits uniform series of autonomous pulsations. As in the autonomous pulsation of the leaflet of *Desmodium gyrans*, the contractile fall of the leaflet is very rapid, while the recovery is comparatively slow. In both rise of temperature increases the frequency of pulsation. The autonomous activity of the *Averrhoa* leaflet may be regarded as 2.5 times slower than that of *Desmodium*.

IV. THE EXCITATORY IMPULSE IN ORDINARY PLANTS

BY

S. C. DAS, M.A., AND B. K. PALIT, B.Sc.

NUMEROUS experiments, carried out at the Institute, have been described elsewhere proving that in sensitive plants like *Mimosa pudica* there are conducting tissues which transmit the excitatory impulse in a manner analogous to the nervous impulse in the conducting nerve of the animal.¹ Accounts of further researches on the subject are also to be found in Vol. vii. of the *Transactions of the Bose Research Institute*.

It would no doubt be of considerable theoretical importance if a wider generalisation could be established by the discovery of parallel effect in ordinary plants. For the purpose of the present investigation we therefore employed young specimens of either the Bean plant (*Vicia Faba*) or of *Vigna Catjang*.

The leaves of these plants are usually regarded as insensitive since, on stimulation, they do not exhibit any marked responsive movement. It will, however, be shown that the moto-excitability of the pulvinus of the leaves and of the pulvinules of the leaflets is not altogether absent, but is so feeble as to be inconspicuous. By the employment of recorders (usually magnifying about 50 times) it is quite possible to detect and record the movement induced in them by either direct or indirect stimulation.

In fig. 22 is seen a semi-diagrammatic representation of certain portions of a young Bean plant. S is the stem, and

¹ Bose, *Nervous Mechanism of Plants* (1926), p. 206.

P the main pulvinus at the junction of the stem and the petiole ; the latter is prolonged to *Pt*, and is shown to bear two lateral and a terminal leaflet. The pulvinules supporting the lateral leaflets are marked as *p*, *p*, and that supporting the terminal leaflet as *p'*. It is to be borne in mind, that the motor organs are the main pulvinus and the pulvinules ; if an excitatory impulse is transmitted by a con-

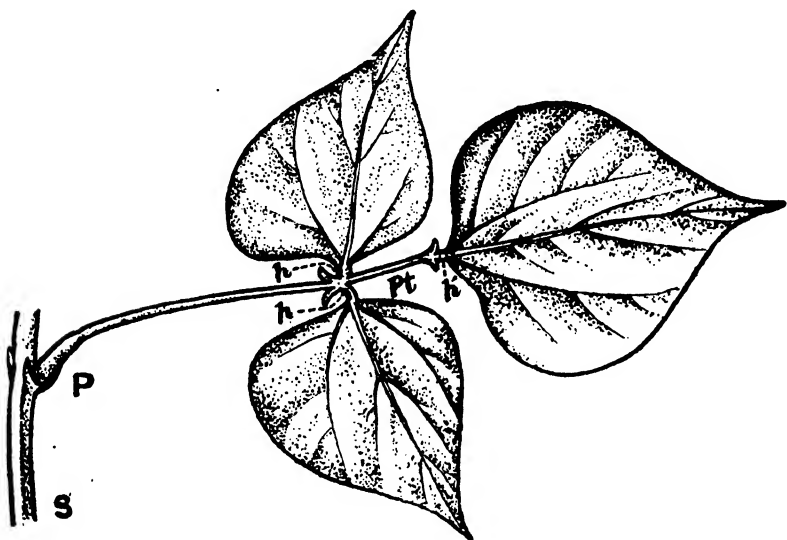


FIG. 22. Diagrammatic representation of petiole-pulvinus preparation of the Bean plant (*Vicia Faba*).

s, the stem ; *Pt*, the prolongation of the petiole ; *P*, the main pulvinus ; *p*, *p*, the pulvinules bearing the two lateral leaflets, and *p'* that supporting the terminal leaflet.

ducting tissue the fact can be verified by the responsive movements of these motor organs which are to be recorded automatically.

Three different methods have been employed to obtain records of the responsive movements and their time-relations. When the movement is sluggish the record is obtained by means of the *Oscillating Recorder* in which the smoked glass plate is made to move to and fro by means of clockwork, the successive dots inscribed being usually at intervals of

15 seconds. For recording moderately quick movements the *Electro-magnetic Tapping Recorder* is employed, the successive dots being from 0.5 to 1 second apart. For the measurement of still shorter intervals of time, as in the determination of the latent period of the motor organ and the velocity of transmission of the excitatory impulse, the employment of the *Resonant Recorder* is essential, the writing lever of which is tuned to make successive dots at intervals of 0.1 second.

The subject of inquiry of the present Paper is in regard to the determination of the responsive characteristics of the motor organ in an ordinary plant as well as the velocity of transmission of its excitatory impulse. The different aspects of the subject will be treated in the following order summarised under the main headings A, B and C.

- A. 1. Determination of the relative moto-excitability of the pulvinus and of the pulvinule.
2. Relative excitability of the upper and the lower halves of the motor organ.
3. The polar action of a constant current in excitation.
4. Effects of Kathode-break and of Anode-make of a constant current.
- B. 5. Effects of direct electrical stimulation of four quadrants of the pulvinus.
6. Definite innervation between the centre and the periphery.
7. Direct and indirect effect of transmitted excitation in *Vigna Catjang*.
8. Direct effect of transmitted excitation in *Erythrina indica*.
9. Indirect effect of transmitted excitation in *Erythrina indica*.
- C. 10. Determination of the latent period of t' , in spite of the fact that t' is taken at the same place as t .
11. Measurement of the velocity of transmission of the impulse, in the pulvinus as taken at the same place as t .
12. Arrest of the excitatory impulse by relative excitatory tonic block.

13. Preferential conduction in the petioles of ordinary plants.
14. The relative velocity of centrifugal and of centripetal impulse in the petiole.

EFFECT OF SEASON IN MODIFYING THE IRRITABILITY OF ORDINARY PLANTS

The plants *Vicia Faba* and *Vigna Catjang*, which were in an optimum condition in spring, were found to be adversely affected by the abnormal heat during the summer months, when the temperature often rose as high as 42° C., which is far above the optimum. In consequence of this, the sensibility of the pulvinus, as well as that of the pulvinule, became considerably diminished, and the conducting power for transmission of excitation to a distance also underwent a great depression. During summer months, moreover, the plants shed their leaves, and it was only after the lowering of temperature during the rains that freshly grown leaves were available for the continuation of experiments on the contractility and the conductivity of plants.

RELATIVE MOTO-EXCITABILITY OF THE PULVINUS AND OF THE PULVINULE

For determination of the relative excitability of the main pulvinus and of the pulvinule, a testing stimulus of similar intensity is to be applied in succession to both. The amplitudes of the two responses furnish data for the comparison.

Experiment 1. *Determination of the relative excitability of the pulvinus and of the pulvinule.*—The pulvinus P and the pulvinule p' of the terminal leaflet (cf. fig. 22) were in

Three subjects subjected to testing electric shocks of the same intensity by means of an induction coil.

When the above experiments showed that the moto-excitability of the pulvinule is very much greater than that of the pulvinus, it was found that the moto-excitability of the pulvinule is very much greater than that of the pulvinus. As the amplitudes of the two responses are the successive ones, they cannot be properly represented in the

same figure for purposes of comparison. In order to get over this difficulty, the magnification of the record of response of the pulvinus was made ten times that of the pulvinule, that is to say, the magnification employed for the pulvinar movement was 50 times, while that of the pulvinule was only 5 times. The relative moto-excitability of the pulvinus and of the pulvinule can then be determined with a fair degree of accuracy by actual measurements of the heights of the two responses, allowance being at the same time made for the different magnifications that have been employed.

As regards the response of the pulvinule it is to be remembered, that being attached to the petiole, any movement of the latter, however produced, would complicate the pure response of the pulvinule. For removing this source of error, the response of the pulvinule to electric stimulation is taken when the petiole is held rigid by tying it to a vertical rod. The records of responses were then taken on a moving smoked glass plate carried by the *Oscillating Recorder*, the plate being maintained in a state of oscillation to and fro at intervals of 15 seconds. The response of the pulvinus to similar testing electric shocks is also taken by the *Oscillating Recorder*.

The results are seen in fig. 23, in which the two records P and *p* are the respective responses of the main pulvinus and of the pulvinule to similar testing electric shocks. In this and in all subsequent records the up-curve represents the fall of the leaf or of the leaflet. The erectile movement, on the other hand, is represented by a down-curve. The stimulus of induction shock was in each case applied at the up-pointing arrow. It will be noted that in P the intervals between successive dots in the contractile up-curve (representing the fall of the leaf) are relatively closer than in the record *p* of the responsive fall of the lateral leaflet, in spite of the fact that the response of the pulvinus was taken at a magnification which was ten times greater than that of the pulvinule. The period of recovery of the pulvinus was also considerably longer. As regards the relative excitability of the two motile organs, the pulvinus and the

pulvinule, it is seen that the heights of the two responses are practically equal, though the pulvinar response was taken at a magnification which was ten times greater than that for the response of the pulvinule.

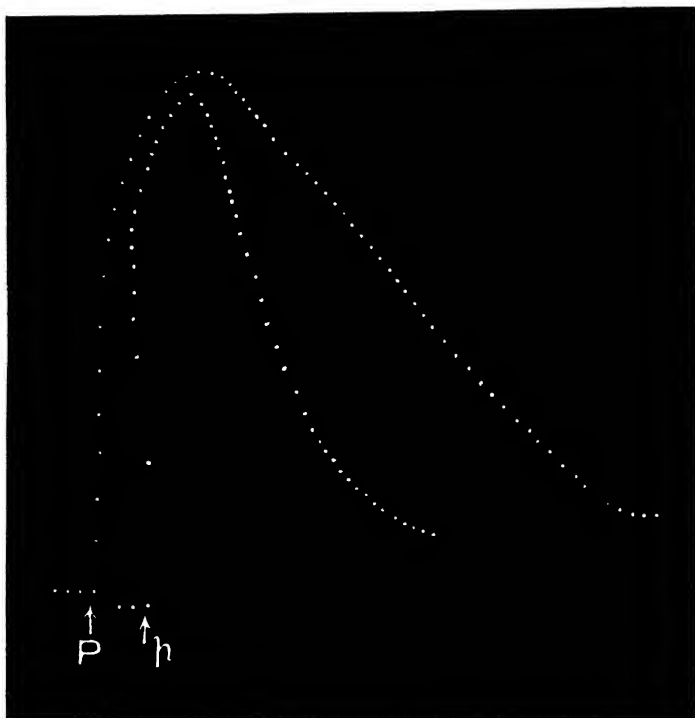


FIG. 23. Responses to direct testing electric shock; P, record of the main pulvinus, and *p* that of the pulvinule (*Vigna Catjang*).

Successive dots at intervals of 15 seconds.

The above results lead to the following conclusion :

- (1) That the moto-excitability of the pulvinus is only about one-tenth that of the pulvinule.
- (2) That the response of the pulvinus is comparatively sluggish, as indicated by its slower contractile reaction and longer period of recovery.

RELATIVE EXCITABILITY OF UPPER AND LOWER HALVES
OF THE MOTOR ORGAN

The responsive fall of the motor organ that has just been described occurred under diffuse stimulation by electric shocks from an induction coil, both the upper and lower halves of the organ being thus excited simultaneously. The question now arises: How is the resultant movement of the fall of the leaf or leaflet produced? Is this due to the reaction of the upper or of the lower half, or is it due to the joint effects of both? The question can only be solved by taking separate records of the effects of local stimulation of the two halves, say, of the pulvinule. For local stimulation the polar action of the sudden starting of a constant current was found to be most convenient. It will presently be shown that excitation is induced by 'make' of the constant current at the Kathodic point where the current leaves the tissue. For carrying out this method of stimulation into practice, the Anodic and Kathodic connections with the plant were suitably made with the two poles of a voltaic battery, the current being suddenly started by means of a tapping key. The Kathodic connection was made either with the upper or with the lower half of the motile pulvinule for the two successive experiments, by means of sharp-pointed platinum electrodes, which slightly entered into the tissue; the Anodic connection was made at a distant indifferent point on the stem.

Experiment 2. *Response of the lower and upper halves of the pulvinule of Vigna Catjang.*—From the records in fig. 24 it will be seen that the contractile reaction of the lower half of the pulvinule, represented by the up-curve, is very vigorous and abrupt, the contraction of the lower half of the leaflet causing *the fall of the leaflet*. The upper half of the pulvinule, which was next subjected to the stimulation of Kathode-make, gave response by contraction of that half of the pulvinule, resulting in an *up-movement of the leaflet*. This, represented by the down-curve, is very sluggish and feeble. The amplitudes of the two responses

are in the ratio of 64 : 6·5, proving the far greater excitability of the lower half of the particular motor organ.

The results prove that the upper and the lower halves of the pulvinule act antagonistically to each other, and that the resultant movement of fall of the leaflet, which occurs

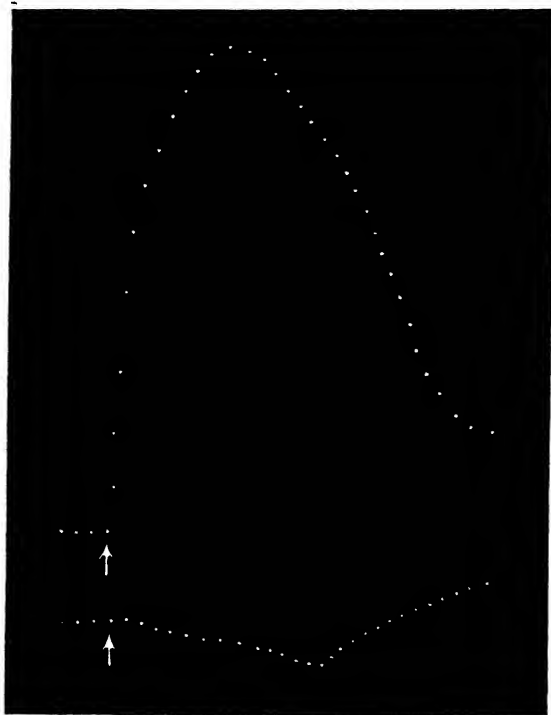


FIG. 24. Contractile responses of the lower half (up-curve) and of the upper half (down-curve) of the pulvinule (*Vigna Catjang*).

Successive dots at intervals of 15 seconds.

under diffuse stimulation, is due to the predominant excitability of the lower half of the organ.

Experiment 3. *Response of the lower and upper halves of the main pulvinus of Vigna Catjang.*—The method of procedure is precisely similar to that in the last case. Here also the lower half of the pulvinus was found to be more

excitable than the upper, the amplitudes of the two responses under similar testing stimulus being in the ratio of 62 : 38. The differential excitability, on which the vigour of the resultant response depends, is thus less in the pulvinus than in the pulvinule. This may be one of the reasons why the pulvinus is relatively less sensitive to diffuse stimulation than the pulvinule.

THE POLAR ACTION OF A CONSTANT CURRENT IN EXCITATION

As a result of numerous experiments carried out in the Institute it has been found that the polar action of a constant current on sensitive plants like *Mimosa pudica* is essentially similar to that in animals.¹ In plants, as in animals, it is the Kathode that induces an excitatory reaction at *make*, while excitation is induced by the Anode at *break*. These characteristic reactions are also demonstrated in the ordinary plant *Vicia Faba*, as in the following :

Experiment 4. *Excitatory reactions on make of Kathode.*—The pulvinule of the leaflet of *Vicia Faba* was employed for the experiment. Suitable Kathodic connection was made with the more excitable lower half of the pulvinule, the Anodic connection being made as usual with a distant indifferent point. As a source of constant current a battery of voltaic cells was employed, the proper electro-motive force being carefully adjusted by means of a potentiometer included in the circuit. The effective E.M.F. was usually found to be about 6 volts. The current could be sent in one direction or the opposite by means of a Reversing Key. The current was suddenly made or broken by means of a second tapping key.

Under the action of Kathode-make there was produced a sudden fall of the leaflet, due to the induced excitatory contraction of the pulvinule.

Experiment 5. *Excitatory reaction by the break of Anode.*—The direction of the current was next reversed and the effect of the Anode-break on the pulvinule was recorded.

¹ Bose, *Nervous Mechanism of Plants* (1926), p. 27.

This gave rise also to an excitatory contraction, resulting in the fall of the leaflet. The characteristic results obtained with an ordinary plant like *Vicia Faba*, by the polar action of a constant current, are thus similar to those obtained with the sensitive plant *Mimosa pudica*.

Since the excitatory effects are brought about by the 'make' of Kathode and the 'break' of Anode, one might expect that, generally speaking, the effect of Kathode would be opposite to that of the Anode. And further the effect of the 'make' would also be opposite to that of the 'break' of the current. Such opposite or antagonistic reactions have not, however, been observed with the highly sensitive plant *Mimosa pudica*. For while the Kathode-make was found to induce in it an excitatory contraction, no effect could be detected at the Kathode-break. Again, while the 'break' of Anode induced an excitatory contraction, the 'make' of the Anode was apparently ineffective.

The question now arises whether the Kathode-break and Anode-make were really ineffective as has been supposed. The following experiments carried out with the sluggish and relatively less excitable *Vicia Faba* have, however, revealed reactions which may prove to be of considerable theoretical importance.

EFFECTS OF KATHODE-BREAK AND OF ANODE-MAKE OF A CONSTANT CURRENT

In the following series of experiments, a complete cycle of responses of an identical specimen of the leaflet of *Vicia Faba* was recorded, suitable electric connections being made as in previous cases. In order to obtain the most perfect result for the whole cycle, the following desiderata had to be carried out in practice. For avoiding fatigue caused by prolonged duration of the current, this is to be maintained for the shortest possible length of time. It is also desirable to obtain separately the pure effect of Kathode-make, of Kathode-break, of Anode-make and of Anode-break. Thus, for example, the effect of short-lived sudden 'make' of Kathode is to be obtained without the result

being complicated by the effect of 'break' that succeeds it. How then is this 'break' effect of the Kathode to be eliminated? In regard to this, it is well known that the polar action is only effective when the current undergoes a *sudden* and not a continuous variation of its intensity. After the brief Kathode-make the disturbing break-effect is eliminated by gradual reduction or withdrawal of the current, by the proper manipulation of the sliding potentiometer which regulates the acting electro-motive force. In this way it is possible to increase the acting E.M.F. continuously from zero to a maximum, or decrease it from a maximum to zero.

Experiment 6. *Responses of the leaflet throughout a complete cycle of electromotive variation.*—The results are described in detail under four following sub-headings.

(a) *Effect of Kathode-make.*—The E.M.F. was adjusted to the effective value by means of the potentiometer. The Kathodic connection was made with the lower half of the pulvinule, and the current suddenly started by pressing the tapping key, which was kept down for a time. This sudden Kathode-make at arrow resulted in a pronounced down-movement of the leaflet, the excitatory *contraction* being indicated by the up-curve Km in fig. 25. The constant current was then *gradually* withdrawn at the second arrow within a circle. This made it possible to obtain the pure effect of Kathode-make, uncomplicated by the effect of the subsequent sudden Kathode-break. The contractile response persisted for a short time even after the withdrawal of the current, after which recovery became practically complete in the course of about 8 minutes.

(b) *Effect of Kathode-break.*—After the natural recovery just described, the E.M.F. was gradually increased by the potentiometer to the required value, the tapping key having been kept pressed down all the while. The key was next raised, causing a sudden interruption of the current, constituting Kathode-break. *This resulted in an up-movement of the leaflet, indicated by the down-curve, Kb in the record, due to the induced expansion of the lower half of the pulvinule* (fig. 26). Here is exhibited the remarkable manifestation

of an *expansion* induced by the Kathode-break, which is antagonistic to the excitatory reaction of Kathode-make.



FIG. 25.

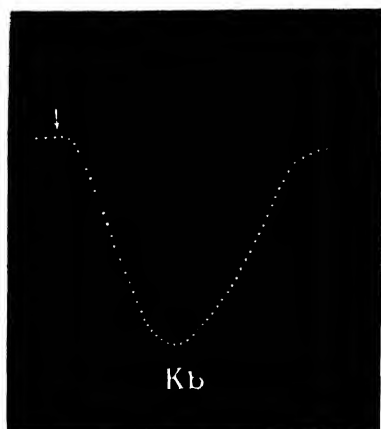


FIG. 26.

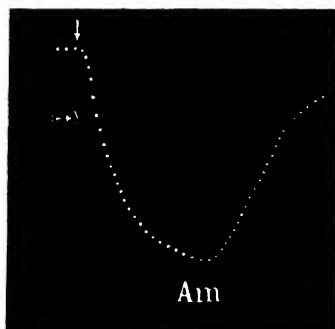


FIG. 27.

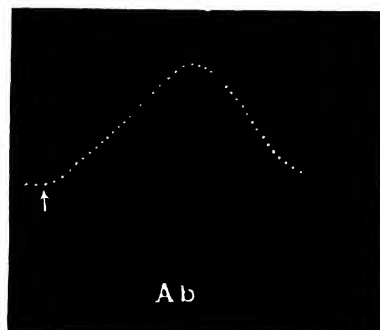


FIG. 28.

FIGS. 25, 26, 27, 28. Records of responses of the terminal leaflet of ordinary plant to the action of a constant current (*Vicia Faba*).

FIG. 25. Km. Response to the action of the make of Kathode.

FIG. 26. Kb. Response to the action of the break of Kathode.

FIG. 27. Am. Response to the action of the make of Anode.

FIG. 28. Ab. Response to the action of the break of Anode.

The arrow within a circle represents cessation of stimulation in these and in other experiments.

As the contractile effect of Kathode-make is excitatory, the opposite expansive reaction of Kathode-break must naturally be regarded as anti-excitatory.

(c) *Effect of Anode-make*.—The direction of the current was next changed by means of the Reversing Key. After adjustment of the proper E.M.F. the current was suddenly started by means of the tapping key, the lower half of the pulvinule being thus subjected to the action of Anode-make. This gave rise to an *expansive* reaction, exhibited by the up-movement of the leaflet, indicated by a down-curve Am (fig. 27). The current was now slowly withdrawn at arrow within a circle for securing natural recovery.

(d) *Effect of Anode-break*.—The procedure is in every way similar to that adopted for the Kathode-break. The sudden break of the Anode resulted in an excitatory contraction and fall of the leaflet, represented by the up-curve Ab (fig. 28). The response is less vigorous than was expected, probably on account of growing fatigue, which resulted from prolonged experimentation with an identical specimen.

The results obtained in the above experiments are highly suggestive; *they prove the antagonistic reactions, not only of the two poles but also of the 'make' and 'break' of the current. The Kathode-make gives rise to contraction, while the Anode-make causes expansion. Moreover, the effect of Kathode-break is opposite to that of Kathode-make, and the effect of Anode-break is opposite to that of Anode-make. Of these the contractile reaction is excitatory, while the expansive reaction is anti-excitatory.*

EFFECTS OF DIRECT STIMULATION OF FOUR QUADRANTS OF THE PULVINUS

Previous researches carried out at the Institute have established the fact that the pulvinus of *Mimosa* is a complex organ, consisting of four quadrants which on local stimulation give rise to certain characteristic movements of the leaf. Thus stimulation of the upper quadrant induces a rectilinear up-movement of the leaf, while stimulation of the lower quadrant causes rectilinear down-movement. Local stimulation of the right quadrant, on the other hand, gives rise to a right-handed torsion, while stimulation of the left quadrant induces a left-handed torsion.

The following experiments will demonstrate that the characteristic responses of ordinary plants to local stimulation of the different quadrants are, in every way, similar to those of the sensitive *Mimosa*.

For local stimulation of the different quadrants it is most convenient to employ the excitatory action of a constant current, brought about by the 'make' of the Kathode.

The necessary Kathodic connection with each quadrant of the pulvinus is made by thrusting into it a fine platinum point, the Anodic connection being made with a distant indifferent area on the stem. The different quadrants are separately stimulated locally by the sudden starting of the constant current.

Experiment 7. *Contractile responses of the lower and upper quadrants of Vigna Catjang*.—The lower quadrant L and the upper quadrant U were locally stimulated in succession by the Kathode-make. For obtaining the record, the petiole was tied by means of a silk thread to the short arm of a writing lever, the magnification produced being fifty times. The curve of response was obtained on a moving smoked glass plate which was kept oscillating to and fro by clockwork, the successive dots being at intervals of fifteen seconds. The response on stimulation of the lower quadrant was a vigorous rectilinear down-movement exhibited as an up-curve, seen in fig. 29. The response of the upper quadrant, on the other hand, is a rectilinear up-movement due to the contraction of that quadrant, and shown as a down-curve in fig. 30.

Experiment 8. *Effect of local stimulation of the right and the left quadrants*.—Stimulation of these quadrants gave rise to torsional responses which were right-handed (clockwise) or left-handed (anti-clockwise) respectively. For obtaining a record, one arm of an L-shaped flattened aluminium wire is tied securely to the petiole, the other arm being attached to the short arm of a magnifying lever.¹ Any other movement of the petiole except the torsional is prevented by a glass hook which encloses the petiole. Stimulation of the right quadrant resulted in a right-handed

¹ Bose, *Nervous Mechanism of Plants* (1926), p. 153.

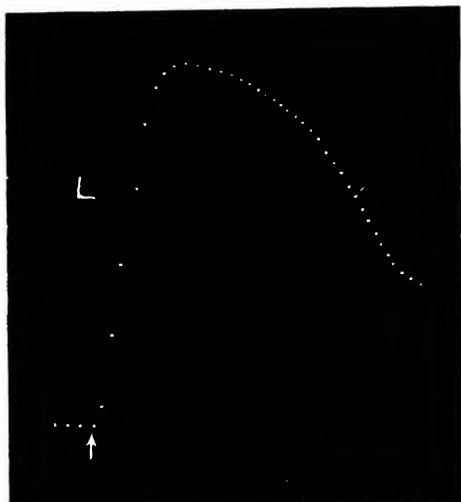


FIG. 29.

FIG. 29. Rectilinear down-response (up-curve) due to contraction of the lower quadrant L of the primary pulvinus under local stimulation of Kathode-make (*Vigna Catjang*).

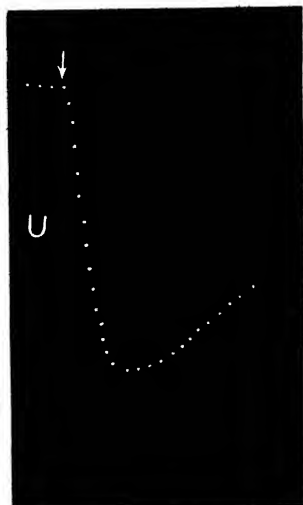


FIG. 30.

FIG. 30. Rectilinear up-response (down-curve) due to contraction of the upper quadrant U by local Kathodic stimulation (*Vigna Catjang*).

Successive dots at intervals of 15 seconds.

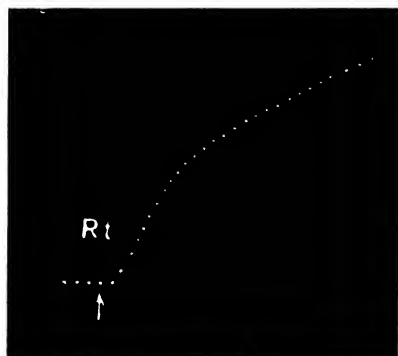


FIG. 31.

FIG. 31. Right-handed torsion Rt by local stimulation of the right quadrant of the pulvinus (*Vigna Catjang*).

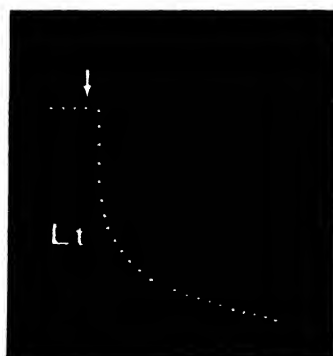


FIG. 32.

FIG. 32. Left-handed torsion Lt by local stimulation of the left quadrant of the pulvinus (*Vigna Catjang*).

Successive dots at intervals of 15 seconds.

torsional movement, Rt, shown as an up-curve in fig. 31; whereas local stimulation of the left quadrant caused the opposite movement of left-handed torsion Lt, indicated as a down-curve (fig. 32).

The characteristic responses of the four quadrants of ordinary plants are thus seen to be similar to those of the sensitive *Mimosa pudica*.

DEFINITE INNERVATION BETWEEN THE CENTRE AND THE PERIPHERY

Experiments will next be described which prove that there is a definite conducting tissue constituting the innervation of plants; the impulse initiated at the central end of the conducting tissue at the different quadrants of the pulvinus are in this way transmitted as an outgoing impulse, causing responsive fall of the corresponding leaflets.

METHOD OF EXPERIMENT

The petiole is held in its normal position in a clamp without undue pressure, a fine sharp pin being slowly introduced into one or the other of the quadrants till the conducting tissue is reached. After a suitable period of rest excitatory impulse is initiated at the different quadrants by making the pin-applicator as the Kathode, the Anode being at a distant indifferent area in the stem.

As already stated, a certain time is allowed for the disappearance of the irritation caused by the prick and the rough handling of the specimen. By means of a tapping key, interposed in the circuit, excitatory impulse is initiated at the central end of the conducting tissue by Kathode-make at each quadrant of the pulvinus. The characteristic effect of the outgoing impulse is then detected and recorded by the responsive fall of the corresponding leaflet.

Experiment 9. *Transmitted excitation by stimulation of left flank or quadrant.*—The centrifugal impulse generated caused after a short time the responsive fall of the leaflet to the left, seen as an up-curve. There was a recovery on the

cessation of the constant current (fig. 33). It was noted in this connection that no such excitatory fall was exhibited by the leaflet to the right or by the terminal leaflet.

The above facts prove that the left quadrant is in conducting communication with the left leaflet, which may therefore be regarded as corresponding to the left quadrant of the pulvinus.

Experiment 10. *Transmitted excitation by stimulation of the right flank or quadrant.*—The outgoing impulse, due to Kathodic stimulation of the right flank of the pulvinus, was



FIG. 33.

FIG. 33. Response of the left leaflet due to transmitted excitation by stimulation of the left quadrant of the pulvinus *Vigna Catjang*.

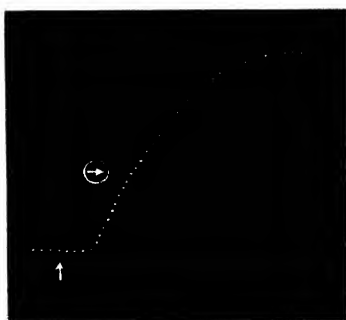


FIG. 34.

FIG. 34. Response of the right leaflet due to transmitted excitation by stimulation of the right quadrant (*Vigna Catjang*).

Successive dots at intervals of 15 seconds.

now found to cause the fall only of the leaflet to the right (fig. 34), which thus corresponds to the right flank of the pulvinus.

Experiment 11. *Transmitted excitation by stimulation of the upper quadrant.*—The impulse generated by the Kathode-make brought about the *erectile* movement of the terminal leaflet, exhibited as a down-curve (fig. 35), evidently due to the excitatory contraction of the upper half of the pulvinule.

Experiment 12. *Transmitted excitation by stimulation of the lower quadrant.*—The impulse generated by the Kathode-make caused the *fall* of the terminal leaflet, shown as an

up-curve (fig. 36), due to the greater and more energetic contraction of the lower half of the pulvinule.

The records of the characteristic effects in the four preceding figures prove that there is a definite conducting communication between the central and peripheral ends of the leaf. They also indicate that the left leaflet corresponds to the left quadrant ; the right leaflet to the right quadrant ;

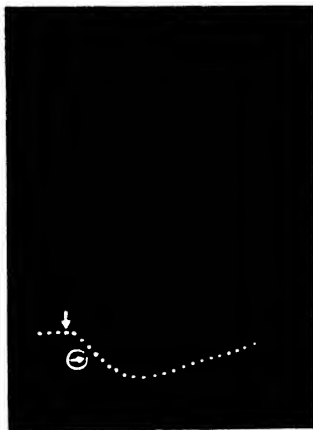


FIG. 35.

FIG. 35. Response of the upper half of the pulvinule of the terminal leaflet by stimulation of the upper quadrant of pulvinus (*Vigna Catjang*).

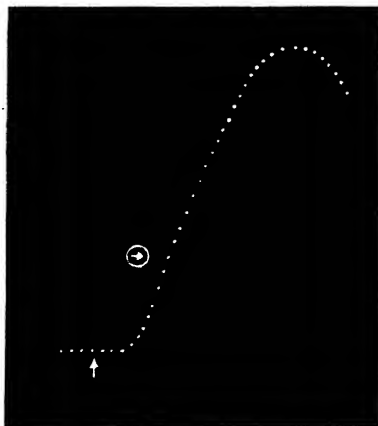


FIG. 36.

FIG. 36. Response of the lower half of the pulvinule of the terminal leaflet by stimulation of the lower quadrant of pulvinus (*Vigna Catjang*).

the upper half of the pulvinule of the terminal leaflet to the upper quadrant ; and the lower half of the pulvinule of that leaflet to the lower quadrant.

DIRECT AND INDIRECT EFFECT OF TRANSMITTED EXCITATION IN *VIGNA CATJANG*

It has been shown that the transmitted outgoing impulse, which directly impinges on the corresponding leaflet, induces a contractile reaction. Since the transmitted impulse acts directly on the motile organ of the particular leaflet, the

reaction may for convenience be regarded as the *direct* effect of the transmitted excitation. Taking a concrete example, the impulse initiated at the left quadrant caused the fall only of the corresponding leaflet to the left; it was also shown that the leaflet to the right *did not exhibit any such contractile fall*. From this result does it follow that there is no effect whatsoever induced on the opposite leaflet to the right? For obtaining an answer to this question, the right leaflet was next attached to the writing lever and the responsive movement, if any, of the right leaflet, due to transmitted impulse from the left quadrant, was recorded.

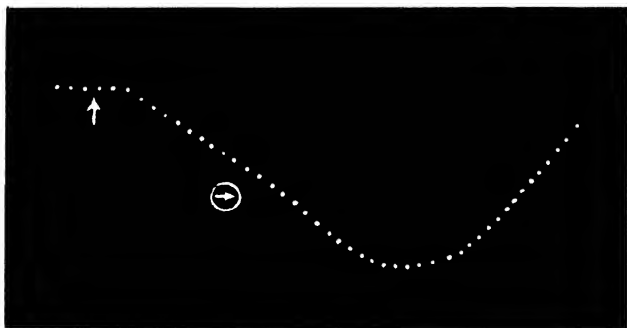


FIG. 37. Effect of Kathodic stimulation of the left quadrant in inducing expansive reaction on the opposite leaflet to the right (*Vigna Catjang*).

Experiment 13. *Effect of the transmitted impulse from left quadrant on the leaflet to the right.*—The excitatory impulse was initiated at the left quadrant in the normal manner, by the Kathode-make, and the effect on the right leaflet detected by means of the automatic record, which indicated the surprising result that the leaflet to the right exhibited *an erectile instead of a contractile movement*. This is clearly seen in fig. 37, in which the down-curve represents the *erectile* movement of the particular leaflet, which is followed by recovery on the cessation of stimulation.

This result was then further verified by a corroborative experiment, in which stimulation by Kathode-make was transferred from the left to the right flank of the pulvinus.

This gave rise to the normal responsive *fall* of the leaflet to the right, while it brought about an expansive *erectile* response of the opposite leaflet to the left.

TRANSMITTED IMPULSE IN *ERYTHRINA INDICA* BY MECHANICAL STIMULATION OF QUADRANTS

In order to establish the universality of definite innervation between the centre and the periphery, parallel ex-

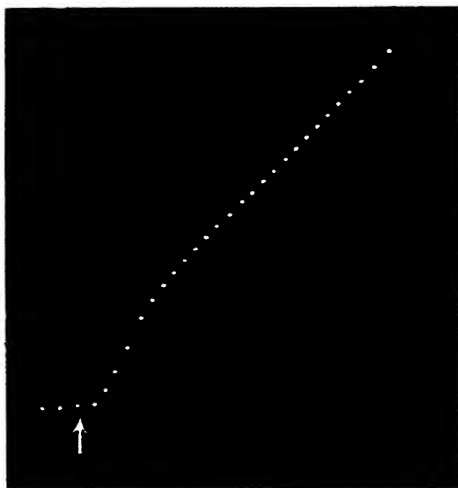


FIG. 38. Response of the left leaflet by mechanical stimulation of the left quadrant of the pulvinus (*Erythrina indica*).

• Successive dots at intervals of 30 seconds.

periments were carried out with a different species of ordinary plants such as *Erythrina indica*. In this the disposition of the lateral and terminal leaflets is similar to that in *Vigna Catjang*. The mode of stimulation employed at the centre was, however, different, being mechanical instead of electrical. For this purpose scratch stimulus was successively applied at the different quadrants.

Experiment 14. *Effect of mechanical stimulation of the left flank or quadrant of Erythrina on the leaflet to the left.*—The transmitted outgoing impulse induced the contractile fall of the leaflet to the left. The record is seen in fig. 38, in

which the contractile fall is shown as an up-curve, demonstrating the continuity of the conducting tissue between the left quadrant and the leaflet to the left.

Experiment 15. *Effect of mechanical stimulation of right flank or quadrant on the leaflet to the right.*—The transmitted

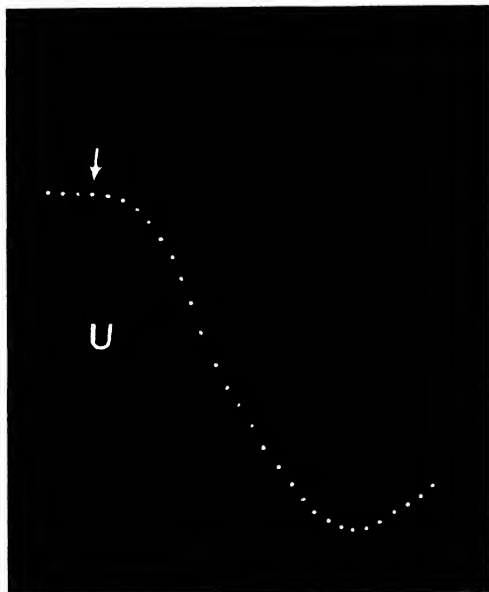


FIG. 39.

FIG. 39. Response U of the upper half of the pulvinule of terminal leaflet by mechanical stimulation of the upper quadrant of pulvinus (*Erythrina indica*).

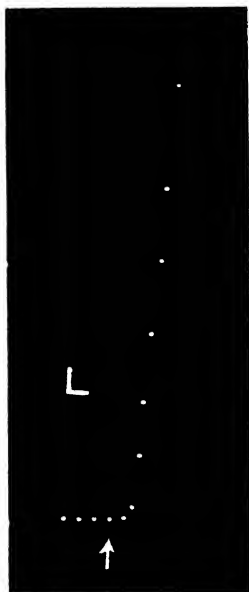


FIG. 40.

FIG. 40. Response L of the lower half of the pulvinule of the terminal leaflet by mechanical stimulation of the lower quadrant of pulvinus (*Erythrina indica*).

Successive dots at intervals of 30 seconds.

impulse induced a fall of the leaflet on the same side, proving that the right quadrant and the right leaflet were in conducting communication with each other.

Experiment 16. *Effect of mechanical stimulation of the upper quadrant on the terminal leaflet.*—The result is very similar to that of experiment on the pulvinus of *Vigna Catjang*. The transmitted impulse from the upper quadrant

induced an up-movement of the terminal leaflet (fig. 39) by the excitatory contraction of the upper half, U, of its pulvinule.

Experiment 17. *Effect of mechanical stimulation of the lower quadrant on the terminal leaflet.*—Mechanical stimulation of the lower quadrant caused a more energetic down-movement by the excitatory contraction of the lower half of the pulvinule, seen in the up-curve of fig. 40.

The results described on the effect of mechanical stimulation on different quadrants of *Erythrina indica* prove once more that a definite conducting communication exists in ordinary plants between the centre and the periphery.

INDIRECT EFFECT OF TRANSMITTED IMPULSE IN *ERYTHRINA INDICA*

It has been shown that while transmitted impulse from a particular quadrant induces contractile response of the corresponding lateral leaflet, its effect on the opposite leaflet is of a different sign, namely, an expansive instead of the normal contractile reaction. This was demonstrated by experiments carried out with the leaf of *Vigna Catjang*, the centrifugal impulse being generated at a particular quadrant of the pulvinus by the Kathode-make (*cf.* Experiment 13). In that experiment the contractile response of the leaflet on the same side, and the contrasted expansive reaction of the leaflet on the opposite side were, however, obtained not simultaneously but in succession, one after the other.

It was now thought desirable to obtain an independent support in verification of the contrasted reaction of the opposite leaflets, the two responses being taken at the same time. For obtaining the necessary records the two lateral leaflets were attached respectively to two different writing levers in a double recorder, so that the records of the two lateral leaflets could be obtained simultaneously.

Experiment 18. *Opposite reactions in the two lateral leaflets by stimulation of a particular quadrant.*—As a typical example the effects of mechanical stimulation of one of the

quadrants, in this case the left, on the two leaflets are shown in the next two figures.

The transmitted impulse, impinging on the leaflet on the same side, gives rise to direct stimulation (D), and causes the contractile fall of the leaflet on that side, *i.e.* the left, shown by the up-curve (fig. 41). The simultaneous record of the leaflet on the opposite right side, which is subjected to

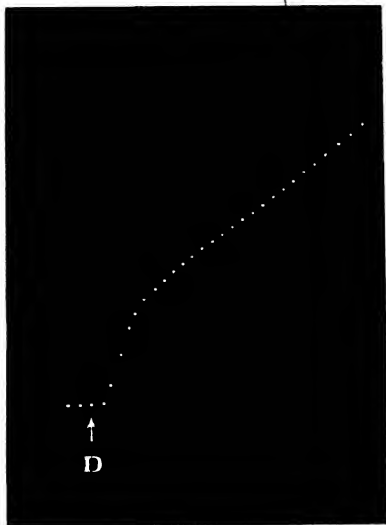


FIG. 41.

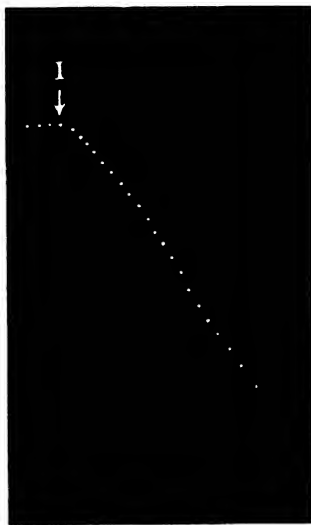


FIG. 42.

FIGS. 41 and 42. Simultaneous records of responses of the two lateral leaflets (*Erythrina indica*) by the impulse generated at the left quadrant.

FIG. 41. Shows contractile response (up-curve) of the left leaflet, due to direct stimulation D.

FIG. 42. Exhibits erectile response (down-curve) of the right leaflet due to indirect stimulation I.

indirect stimulation (I), exhibits an erectile response shown by the down curve (fig. 42).

The explanation for the opposite effects induced in the two leaflets has already been given, and may be briefly recapitulated once more. The results described have offered definite proof of the existence of conducting communication between each lateral quadrant of the pulvinus

with the corresponding leaflet on the same side. The excitatory impulse initiated at a particular lateral quadrant of the pulvinus thus impinges *directly* on the pulvinule on the same side, inducing its normal excitatory fall. There is, however, no direct communication between a lateral quadrant and the leaflet on the opposite side; hence the transmitted impulse can only act indirectly on it.

It has been shown elsewhere¹ that :

- (1) The effect of all forms of *Direct* stimulation is a diminution of turgor, contraction, and the negative mechanical response of the fall of the leaf or leaflet.
- (2) The effect of *Indirect* stimulation, on the other hand, is an increase of turgor, expansion and the positive mechanical response of erection of the leaf or leaflet.

The remarkable opposite reactions exhibited in the two lateral leaflets by an identical stimulus are thus fully accounted for from the above generalisation.

The next problem is the exact determination of the velocity of the excitatory impulse in ordinary plants. The arrival of the excitatory impulse at the distant motor organ is detected by its responsive fall. For the exact measurement of the speed of the excitatory impulse it is necessary to determine the period of transmission, the distance through which the impulse is transmitted and the latent period of the motor organ itself.

DETERMINATION OF THE LATENT PERIOD OF THE MOTOR ORGAN

The latent period of the motor organ is determined from the time-interval between its direct stimulation by induction shocks and the resulting response. This time-interval is found from the automatic record given by the *Resonant Recorder* on a fast-moving smoked glass plate, the successive dots in the record being at intervals of 0.1 second.

¹ Bose, *The Nervous Mechanism of Plants* (1926), p. 94.

Experiment 19. *Determination of the latent period of the pulvinule of Vicia Faba.*—The electric shock was applied directly on the organ at the moment marked with a vertical line. Record given in fig. 43 shows that there is an interval between the stimulus and the response, a sharp flexure of the curve occurring after the 7th dot. The latent period of the pulvinule is, therefore, 0.7 second. This is about seven times slower than the latent period of the more excitable pulvinus of *Mimosa pudica*.

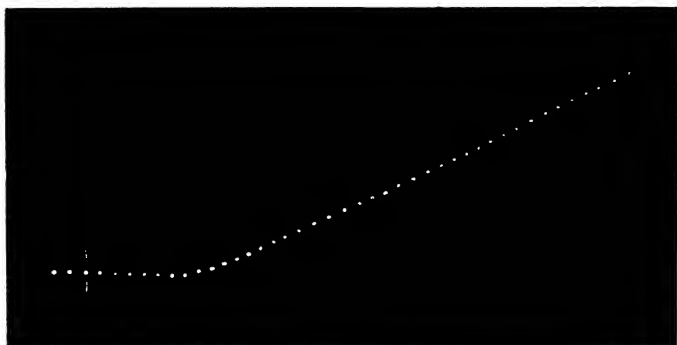


FIG. 43. Record of latent period of the pulvinule of *Vicia Faba*.
Successive dots at intervals of 0.1 second.

The latent period of the pulvinule of *Vigna Catjang* was found to be of the same order as that of *Vicia Faba*.

MEASUREMENT OF THE VELOCITY OF THE EXCITATORY IMPULSE

For the determination of the velocity of the impulse, the middle portion of the petiole *Pt* (cf. fig. 22) is employed. It is then possible to determine the arrival of the centripetal impulse by the fall of one of the lateral leaflets; the centrifugal impulse is, on the other hand, detected by the fall of the terminal leaflet. The excitatory impulse is generated by electric shocks from an induction coil, the effective intensity of which is maintained constant in successive experiments. The impulse in the conducting tissue of the

animal travels in both directions outwards and inwards. The same is found to be true in regard to the conducting tissue of the plant.

The apparatus for determination of velocity of transmission of excitation is the *Electro-magnetic Tapping Recorder*, by which successive dots are inscribed at intervals of 0.5 second. The writing lever gives a magnification of 50 times. The precise moment of application of the stimulus is marked in the record by a vertical line. The record obtained shows that there is a definite interval between the application of the stimulus and the responsive fall of either the terminal or one of the lateral leaflets. The arrival of the impulse at the distant motor organ is indicated by the abrupt flexure of the curve upwards. The time-interval T obtained from the record for the transmission of excitation through the distance d consists of the sum of two factors, namely, the true time of transmission t plus the latent period L of the motor organ itself.

$$T = t + L$$

The velocity of transmission V is found by dividing the distance d , through which the impulse is transmitted, by the true time of transmission t :

$$V = \frac{d}{t} = \frac{d}{T - L}$$

Experiment 20. *Determination of the velocity of centrifugal impulse in the petiole of Vicia Faba.*—A middle-aged leaf was chosen for the determination of the velocity of the outgoing or centrifugal impulse. The petiole of the leaf to be experimented upon was securely held in a vertical support, so that any accidental movement of the leaf did not vitiate the result. The terminal leaflet was attached by a fine silk thread to the short arm of the recording lever, the magnification employed being 50 times. The record, as already stated, was obtained by the *Electro-magnetic Tapping Recorder*, the successive dots being at intervals of 0.5 second. Electrical stimulus was applied on the portion of the petiole Pt at a distance of 10 mm. from the terminal pulvinule.

The intensity of the shock, as well as its duration, was kept constant for all the experiments.

From the curve (fig. 44) it will be seen that the response is initiated at the fifth dot, or 2.5 seconds after stimulation. The latent period of the motile organ has already been found to be 0.7 second (*cf.* Expt. 19). The velocity of the excitatory impulse in the specimen is therefore :

$$V = \frac{d}{T - L} = \frac{10}{2.5 - 0.7} = \frac{10}{1.8} = 5.6 \text{ mm. per second.}$$

Experiment 21. *Velocity of centrifugal impulse in the petiole of Vigna Catjang.*—A parallel experiment was carried out with *Vigna Catjang*; the latent period of the pulvinule

FIG. 44.

FIG. 44. Record of response to centrifugal impulse in the petiole of *Vicia Faba*.

FIG. 45.

FIG. 45. Record of response to centrifugal impulse in the petiole of *Vigna Catjang*.

Successive dots at intervals of 0.5 second.

of the plant is, as already stated, 0.7 second. The total time of transmission, as counted from the moment of application of stimulus indicated by the vertical line, is 2.0 seconds (fig. 45). Hence the velocity

$$V = \frac{10}{2 - 0.7} = \frac{10}{1.3} = 7.7 \text{ mm. per second.}$$

The velocity is, generally speaking, greater in *Vigna Catjang* than in *Vicia Faba*. The result is, however, modi-

fied to a certain extent by the vigour of the individual specimen; by the prevailing temperature; and by the favourable or unfavourable condition of the season.

The following is a tabular statement of typical results.

TABLE I.—VELOCITY OF THE CENTRIFUGAL IMPULSE IN *VICIA FABA* AND IN *VIGNA CATJANG*

Specimen	Length of transmission d	Total time T	Actual time t	Velocity V
<i>Vicia Faba</i>	10 mm.	2.5 sec.	1.8 sec.	5.6 mm. per sec.
" "	15 "	3.0 "	2.3 "	6.5 " " "
" "	12 "	2.5 "	1.8 "	6.7 " " "
<i>Vigna Catjang</i>	9 "	2.0 "	1.3 "	7.0 " " "
" "	10 "	2.0 "	1.3 "	7.7 " " "
" "	10 "	1.5 "	0.8 "	12.5 " " "

The average velocity of centrifugal impulse in the petiole of *Vicia Faba* may be taken as 6.3 mm., whereas in *Vigna Catjang* it is about 9 mm. per second.

In order to prove that the impulse is essentially physiological, the effect of electro-tonic block in the arrest of the impulse is described below.

ARREST OF THE EXCITATORY IMPULSE BY THE ELECTRO-TONIC BLOCK

One of the crucial tests in demonstration of the physiological character of the transmitted impulse is the arrest

of the impulse by the interposition of an electro-tonic block on the path of conduction of excitation. The nervous impulse in an animal is arrested by the interposition of such a block, the conduction being restored on the cessation of the electro-tonic current. Similar effect has been demonstrated in regard to the transmitted impulse in *Mimosa pudica*.¹

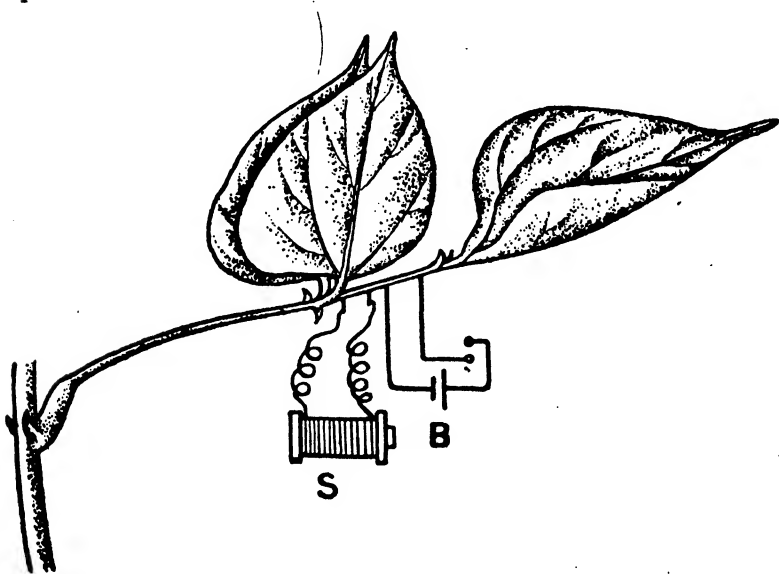


FIG. 46. Diagrammatic representation of the method of electro-tonic block.

s, the secondary coil for initiation of excitatory impulse; the electro-tonic block is interposed in the path of conduction by constant current from battery B.

Experiment 22. *The electro-tonic block*.—The physiological character of transmitted impulse in ordinary plants is demonstrated by the test of the electro-tonic block. The method employed is diagrammatically shown in fig. 46, which consists of the blocking electric current from a battery B, interposed in the path of conduction of the centrifugal impulse, generated by induction shocks from a secondary coil S. In this particular experiment the electric

¹ Bose, *The Nervous Mechanism of Plants* (1926), p. 31.

stimulus was applied on the petiole, at a distance of 15 mm. from the responding terminal leaflet. The record of the velocity of impulse without block is first obtained; the electro-tonic block is then applied, and the effect recorded. Finally the block is removed and the result shown in the subsequent record. In the series of curves given in fig. 47,

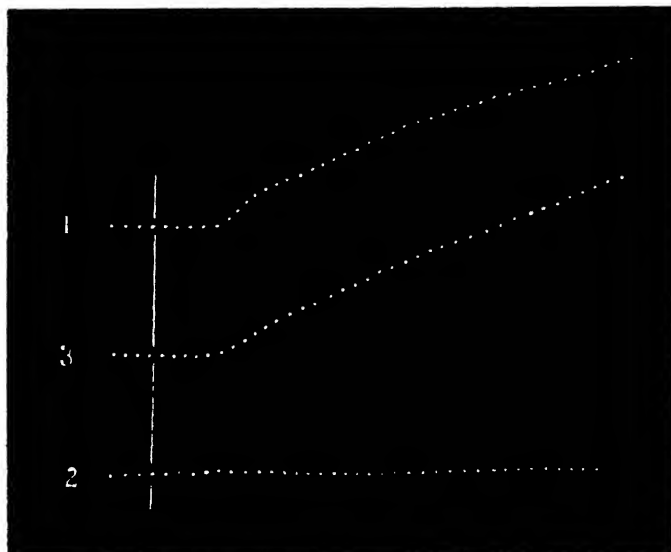


FIG. 47. Series of records demonstrating the effect of electro-tonic block 'on' or 'off' on transmission of excitation in the petiole of *Vicia Faba*.

1. Record of the normal velocity of the impulse.
2. Arrest of the impulse by blocking current.
3. Restoration of the impulse after removal of the electro-tonic block.

Successive dots at intervals of 0.5 second.

the first record indicates the normal transmission of the impulse. The velocity of the transmission as deduced from the record is in this case about 8 mm. per second. The interposition of the electro-tonic block, however, completely arrested the outgoing impulse, as seen in series (2) of the record. Finally, on the removal of the block the impulse was found to be transmitted without any hindrance, as

seen in series (3), the velocity of the transmitted impulse being practically the same as at the beginning.

The effect of the electro-tonic block in arresting the impulse during the period of maintenance of the blocking current, and the restoration of the power of conduction after the removal of the block, prove that the transmission in the plant is essentially a physiological process analogous to the nervous transmission in the animal.

PREFERENTIAL CONDUCTION IN PETIOLES OF ORDINARY PLANTS

In sensitive plants like *Mimosa*, it has been shown that the velocity of transmission in the petiole is unequal in

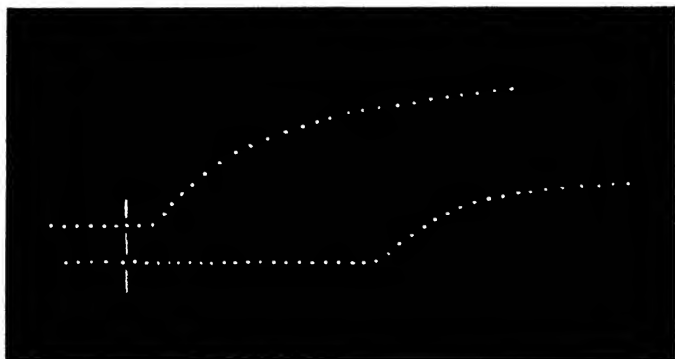


FIG. 48. Records of velocities of transmission in *Vigna Catjang* in the centrifugal (upper curve) and in the centripetal direction (lower curve).

Successive dots at intervals of one second.

centrifugal and in centripetal directions, the centrifugal velocity being considerably higher than the centripetal.¹

In carrying out parallel investigations with ordinary plants, the electrical stimulus was applied midway in the portion of petiole *Pt* (*cf.* fig. 22), so that the distance to be traversed in the two directions was equal, say, 10 mm. in each case.

Experiment 23. *Relation between the velocities in centri-*

¹ Bose, *The Nervous Mechanism of Plants* (1926), p. 48.

fugal and centripetal directions.—The following is an account of an experiment carried out with *Vigna Catjang*, the record of which is given in fig. 48. This particular specimen was in a somewhat subtonic condition and the velocity of transmission was comparatively slow. The time required for transmission of the impulse in the centrifugal direction was 3 seconds. After making allowance for the latent period of the leaflet :

$$\begin{aligned}\text{The centrifugal velocity} &= \frac{d}{T - L} = \frac{10}{3 - 0.7} \\ &= 4.3 \text{ mm. per sec.}\end{aligned}$$

The time for transmission through 10 mm. in a centripetal direction was, however, much longer, being 21 seconds.

$$\begin{aligned}\text{The centripetal velocity} &= \frac{10}{21 - 0.7} \\ &= 0.48 \text{ mm. per sec.}\end{aligned}$$

As in the sensitive plant *Mimosa*, so also in the ordinary plant, the velocity of transmission of impulse in the centrifugal direction is very much greater than in the centripetal. In certain instances this difference is so great that the propagation in the centripetal direction is practically absent; the conduction in such cases may therefore be regarded as of an irreciprocal character.

TABLE II.—THE RELATIVE VELOCITY OF CENTRIFUGAL AND CENTRIPETAL IMPULSE IN THE PETIOLE OF ORDINARY PLANTS

Specimen	Centrifugal velocity	Centripetal velocity
<i>Vicia Faba</i> . .	5 mm. per sec.	0.20 mm. per sec.
<i>Vigna Catjang</i> .	4.3 " " "	0.48 " " "
<i>Vicia Faba</i> . .	5.8 " " "	absent
<i>Vigna Catjang</i> .	7.0 " " "	absent

In the foregoing table are given a few typical results obtained with the ordinary plants *Vicia Faba* and *Vigna Catjang*, which prove that in all cases the centrifugal velocity is considerably higher, and that in certain cases the centripetal conduction is practically absent ; the power of transmission of excitation being in such cases irreciprocal, occurring only in the centrifugal direction.

SUMMARY

An important generalisation is established by the demonstration, that the motor mechanism is essentially similar in sensitive and in ordinary plants.

The four quadrants of the main pulvinus of an ordinary plant act similarly to those of the sensitive *Mimosa pudica*. When the upper quadrant is directly stimulated by an electrical or a mechanical stimulus, the response is a rectilinear up-movement of the leaf. When the lower quadrant is stimulated, there is a more energetic rectilinear down-movement. Stimulation of the left flank induces a left-handed torsion ; that of the right flank gives rise to a right-handed torsion.

The motor organ of the leaf or leaflet is shown to be unequally excitable on the upper and lower halves, the lower half being the more excitable. The resultant movement on diffuse stimulation is due to the predominant contraction of the lower half of the organ.

The terminal or the lateral leaflets of ordinary plants, such as *Vicia Faba*, *Vigna Catjang* and *Erythrina indica*, serve as indicators for the detection of the impulse that reaches the motor organ from a distance.

Polar excitation of the plant-tissue by a constant electrical current due to the Kathode-make and the Anode-break gives rise, as in the animal tissue, to a contractile reaction.

In the highly sensitive *Mimosa*, the effects of Kathode-break and of Anode-make cannot be detected. Their effects have, however, been demonstrated in the relatively sluggish and less excitable ordinary plants. The Kathode-break

induces in these plants an expansive or anti-excitatory reaction ; similar is the effect of Anode-make.

The innervation by which the central end of the leaf is in conducting communication with the periphery, is demonstrated by the excitatory impulse initiated at each of the quadrants, causing the fall of particular leaflets borne by the petiole. Each lateral leaflet at the periphery corresponds to the lateral quadrants on the same side ; the left leaflet thus corresponds to the left quadrant and the right leaflet to the right quadrant.

Though the effect of transmitted impulse from a lateral quadrant is a contractile fall of the leaflet on the same side, yet the same impulse is found to induce an expansive reaction and erectile movement of the opposite leaflet. The explanation of this difference is found from the fact that the transmitted impulse impinging *directly* on the motor organ induces a contractile fall, while the *indirect* effect of the impulse is an expansive erectile movement of the leaflet.

As regards the nature of the transmitted impulse in plants, it is well known that the nervous impulse in the animal is arrested by the interposition of an electro-tonic block, the conduction being restored on the cessation of the electric current. The transmission of the impulse in both sensitive and ordinary plants can likewise be repeatedly arrested or restored by the alternate application and removal of the electro-tonic block. These facts offer conclusive proof that the process of transmission of excitatory impulse in the plant is essentially similar to that of the nervous impulse in the animal.

There is a preferential direction of propagation of the impulse in plants, the velocity in the centrifugal direction being considerably greater. In certain cases the difference is so great that the conduction becomes irreciprocal, occurring only in the centrifugal direction.

V.—INVESTIGATIONS ON THE EFFECT OF EXTERNAL STIMULUS ON DIAMETRIC GROWTH OF STEMS

BY

A. GUHA-THAKURTA, C.S.AG.C., AND B. K. DUTT, B.Sc.

THE difficulty of investigations on the effect of external stimulus and of changes of environment on the growth of plants arises from the extreme slowness of growth. The average rate of the longitudinal growth is about $\frac{1}{100,000}$ inch per second, a length which is half that of a single wave of sodium light. For purposes of measurement of growth in length, auxanometers, magnifying about 20 times, have generally been employed. But even with such a magnification it takes several hours to measure the normal rate of growth and the change induced in it by the action of a particular stimulus. The normal rate of growth is, however, affected by changes in the environmental condition, which cannot be maintained constant for any great length of time. In investigations on the effect of a given mode of stimulation on growth the results are liable to be vitiated by changes in the environment, which inevitably occur during a lengthy period.

The only means of eliminating this difficulty is by reducing the period of the experiment to a comparatively short time, during which the external condition can be maintained approximately constant. The reduction of the period of experiment can be effected by devising a method of exceptionally high magnification; to secure perfect results it is also necessary to invent an automatic method for record of growth, so that personal errors of observation can be completely eliminated.

These requirements have been fulfilled by the *High Magnification Crescograph* devised by Sir J. C. Bose, which gives an automatic record of the rate of growth at a magnification varying from three to ten thousand times.¹ In regard to the absolute determination of the rate of growth, since it is exceedingly feeble, the micron or μ is adopted as the unit of length (the micron being a millionth of a metre, or 0.001 mm.), and a second as the unit of time.

By the employment of the *High Magnification Crescograph*, it has thus been possible, not only to determine the normal rate of longitudinal growth, but also the minute variation induced in it under the action of various modes of external stimulation. A concrete example will give an idea of the extraordinary small rate of growth, the slight variation of which has to be measured. The rate of longitudinal growth of a particular plant was found to be of the order of 0.5 μ per second. By the method just described, the effect of light in inducing variation of the rate of longitudinal growth has been determined with great accuracy. Photic stimulation under normal condition has thus been found to induce an incipient contraction of the growing tissue resulting in the retardation of the rate of longitudinal growth. Since growth occurs not only in a longitudinal but also in a transverse or diametric direction, the question next arises : What is the effect of diverse modes of stimulation on diametric growth? Would it be possible to obtain an accurate and an almost instantaneous record of the normal rate of diametric growth and of the characteristic changes induced under external agents?

The solution of the problem is surrounded by almost insurmountable difficulties. One of these arises from the fact that the diametric growth of the stem is about 50 times slower than that of the longitudinal growth; coupled with this is the additional difficulty that the movement to be recorded here is not vertical but lateral.

¹ Bose, *Proc. Roy. Soc., B.*, vol. 90 (1919), p. 365.

THE DIAMETRIC GROWTH RECORDER

The various difficulties of the problem have been successfully removed by the particular device of the *Diametric Recorder* for growth, the principle of which is similar to that of the apparatus devised by Sir J. C. Bose for recording infinitesimal contraction of cells.¹ The growing region of a stem is placed between a V-shaped fixed horizontal piece V and a movable horizontal lever L. Diametric growth-

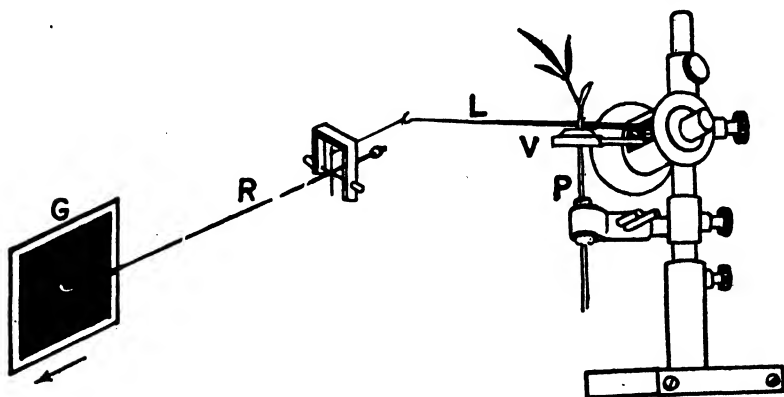


FIG. 49. The Diametric Growth Recorder.

v, fixed horizontal piece ; L, movable primary lever. The second recording lever R, much longer than represented, produces further magnification. The figure shows method of recording diametric growth in a young internode of the plant P, the lower portion of which is not shown. The curve is inscribed on an oscillating smoked glass plate.

expansion moves the lever outwards, whereas contraction moves it inwards. The magnification produced by the primary lever L is slight ; but the total magnification can be greatly increased by means of a second recording lever R (fig. 49). The first lever produces a magnification of 20 times, while the second produces 100 times that of the first, the total compound magnification being thus 2000 times. The writing lever makes successive dots on a moving smoked glass plate which is kept oscillating backwards and forwards

¹ Bose, *Life Movements of Plants* (1919), p. 257 ; *Growth and Tropic Movements of Plants* (1929), p. 72.

at intervals of 45 seconds by means of clockwork, thus giving a dotted record. In reproducing the record, alternate dots have been omitted to prevent overlapping. The distance between successive dots in the record reproduced, therefore, represents a magnified diametric growth which occurs in the course of 90 seconds.

METHOD OF BALANCE

The sensitiveness of the above method is greatly enhanced by the Method of Balance, in which the rate of diametric growth is exactly balanced by a compensating movement in the opposite direction. This is secured by a suitable modification of the device described elsewhere.¹ When the diametric growth is exactly balanced, the record is a horizontal line instead of an ascending curve as in the normal record. The method of balance is found to be extremely sensitive, and the minutest change induced in the rate of growth by the action of an external stimulus is at once detected by the upsetting of the balance and recorded as either an up- or a down-curve. It is thus possible to detect with unprecedented accuracy, not only the direct and after-effects of a particular stimulus, but also the short-lived preliminary variation in the rate of growth, which may occur immediately on the impact of the stimulus. In the records of balanced growth the up-curve indicates an enhancement of the rate of diametric growth above the normal, while the down-curve represents an induced depression below the normal.

The investigations will be taken up in the following order :

1. Effect of light on actively growing young internode of thin stems.
2. Effect of light on actively growing young internode of thick stems.
3. Effect of age of the internode on the response.
4. Effect of electric stimulus on diametric growth.

¹ Bose, *Motor Mechanism of Plants* (1928), p. 131; and also *Growth and Tropic Movements of Plants* (1929), p. 22.

5. Effect of direct and indirect stimulation on diametric growth.
6. Effect of ascending and descending constant electric current on diametric growth.
7. Phototropic curvature under the action of unilateral light.

EFFECT OF LIGHT ON DIAMETRIC GROWTH OF THIN STEMS IN *CAJANUS INDICUS*

For investigations of the effect of light on thin and on thick stems, *Cajanus indicus* was employed as representative of the former, while *Antirrhinum* represented the latter. These plants were found to be specially suitable, since the effect of mechanical irritation, caused by handling during mounting of these specimens for experiment, disappeared after a relatively short period of rest. This was not the case with the majority of other plants, the after-effect of mechanical irritation in which persisted for a considerable length of time. The selected specimens were carefully removed from the soil, taking special care that the roots were not injured. The plant was then placed with its root in water, and suitably mounted in the apparatus. In order that the effect of mechanical irritation, as well as that of previous action of light due to exposure in the open might disappear, the specimen was placed in a dark room for nearly two hours before the commencement of the experiment.

For investigations on the action of photic stimulus on diametric growth, a beam of light from an arc lamp was thrown on the plant, a parallel-sided trough filled with alum solution being interposed in the path of the beam for absorption of the heat rays. Two inclined mirrors were suitably placed behind the plant, so that all sides of the organ could be simultaneously acted upon by light.

The investigations were first carried out on the effect of light on the region of active growth in the young internode.

Experiment 1. *Effect of light on region of active growth in Cajanus.*—The series of illustrations given in fig. 50 are : the record of normal growth ; that of growth under condition

of balance ; the induced modification under photic stimulus ; finally the after-effect on withdrawal of light. The ascending curve N, seen at the beginning, is the normal record of diametric growth which is found from calculation to be 0.0076μ per second. The horizontal record B is then taken under condition of balance, a short gap separating the normal from the balanced record. On application of light on the growing region of the young internode, at the moment marked with an arrow, the record shows, in the course of a short time, an upsetting of the balance *downwards*, indicative

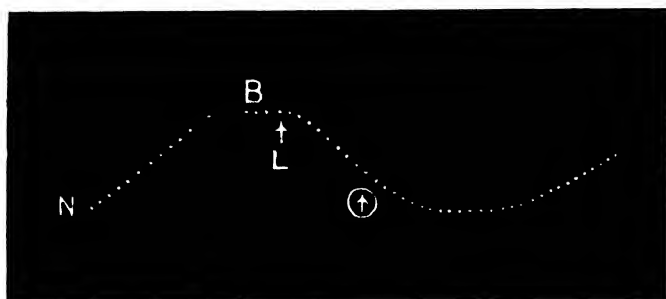


FIG. 50. Effect of light on the diametric growth of *Cajanus*. N, record of normal rate ; B, balanced horizontal record. Light L applied at arrow and withdrawn at arrow within a circle (see text).

Successive dots, reproduced, unless stated to the contrary, are at intervals of 90 seconds.

of retardation of the rate of diametric growth. The latent period, *i.e.* the time which intervened between the application of stimulus and the resulting variation in the rate of growth, is in this case found to be 2.25 minutes.

The light was then withdrawn at the moment marked by the arrow within a circle. The after-effect of light in retardation of growth was found to persist for 13.5 minutes. After this the growth became for a time restored to its original rate, as indicated by the horizontal curve which is parallel to the balanced rate B. There is, however, a further change which occurs later ; for after a period of horizontality, *i.e.* restoration of the original rate of growth,

EFFECT OF EXTERNAL STIMULUS ON DIAMETRIC GROWTH 91

there is a short-lived ascending curve, which indicates a transient acceleration of growth above the normal. This transient acceleration proves that the vigour or tonic condition of the plant is, to a certain extent, improved by the absorption of light.

The following is a tabular statement of typical results obtained with stems of four specimens of *Cajanus*.

TABLE I.—MODIFICATION OF DIAMETRIC GROWTH UNDER LIGHT APPLIED AT ACTIVE REGION OF *CAJANUS*

Number of specimen	Normal rate of growth per second	Latent period of response	Responsive variation of growth
1	0.0060 μ	3.0 minutes	Retardation
2	0.0070 μ	3.0 "	"
3	0.0076 μ	2.25 "	"
4	0.0080 μ	1.5 "	"

It is thus seen that light incident on the active region of growth in a thin internode of *Cajanus* induces a retardation of the rate of diametric growth. The next investigations were on the effect of light on thicker stems of *Antirrhinum*.

EFFECT OF LIGHT ON DIAMETRIC GROWTH OF THICK STEMS OF *ANTIRRHINUM*

The method of experiment is similar to that with *Cajanus*. After taking the normal record of diametric growth the horizontal record under condition of balance is obtained. The effect of light is found from the direction of the upsetting of the balance.

Experiment 2. *Photic stimulation in modification of diametric growth of Antirrhinum*.—The curve of diametric growth N before balance is seen in the first part of fig. 51. The absolute rate of growth is 0.01μ per second. After obtaining the balanced horizontal record B, light is applied at arrow. This caused an upsetting of the balance *upwards*, instead of *downwards*, as in *Cajanus*.

In the thick stem of *Antirrhinum* the stimulus of light thus causes an *acceleration* of the rate of growth in contrast with the *retardation* in the thin stem of *Cajanus*. This

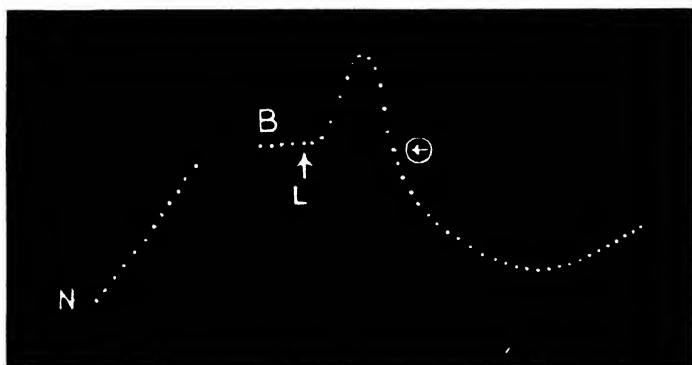


FIG. 51. Effect of light L on diametric growth of *Antirrhinum*. Preliminary acceleration followed by retardation. N, record of normal rate; B, balanced horizontal record; light L applied at arrow and withdrawn at arrow within a circle (see text).

acceleration in *Antirrhinum* is, however, transformed, under continued action of light, into normal retardation as indicated by the reversal of the curve downward. The retardation persisted for a time even on the withdrawal of light at arrow within a circle. The normal rate of growth is then gradually restored, as seen by the resumption of the horizontality of the curve of balance; this restoration is often followed, as in *Cajanus*, by a transient acceleration of the rate of growth above the normal.

Numerous experiments carried out with stems of *Antirrhinum* gave similar results, of which four examples given below may be taken as typical.

TABLE II.—EFFECT OF LIGHT ON DIAMETRIC GROWTH OF *ANTIRRHINUM*

Number of specimen	Normal rate of growth per second	Responsive variation of growth
1	0.008 μ	Acceleration followed by retardation
2	0.009 μ	do.
3	0.009 μ	do.
4	0.010 μ	do.

A certain characteristic difference is thus seen to exist in the responses of *Cajanus* and of *Antirrhinum*; in the former there is a retardation of diametric growth, which occurs within a comparatively short time, whereas in the latter the normal retardation is preceded by a preliminary acceleration of the rate of growth.

In trying to find out the cause of the difference it was found that while in *Cajanus* the layer of actively growing cambium cells is situated at a position relatively near the surface, a similar layer of actively growing cells in *Antirrhinum* is at a greater depth below the surface. It would thus appear that in *Cajanus* the excitation initiated at the surface by the stimulus of light would reach the responsive layer of growth in a relatively short time, and induce the incipient contraction and retardation of growth; stimulation in this case may therefore be regarded as more or less *direct*.

But in *Antirrhinum* the excitation would take a very much longer time to percolate through the greater depth of the intervening tissue; the stimulation in thick stems of *Antirrhinum* may therefore be regarded as *indirect*. It has

been shown elsewhere,¹ that while direct stimulation induces, in general, a retardation of the rate of growth, indirect stimulation enhances the rate. This fact is further verified by results demonstrated by Experiments 7 and 8, described later in this chapter. The preliminary acceleration of diametric growth in *Antirrhinum* can thus be explained from the above consideration; it is, however, to be noted, that even in this case the excitation initiated near the surface reaches the responding layer later and causes the normal retardation of diametric growth. The complete reaction in *Antirrhinum* is therefore a preliminary positive acceleration followed by a negative retardation of growth.

EFFECT OF AGE OF INTERNODE ON RESPONSE

Growth is most active in young internodes of the plant, while it undergoes a continuous decline towards the older internodes below, till there is a total cessation of growth. In illustration of this the two following examples may be cited. The average rate of diametric growth in the young internode of *Cajanus* comes out, from the special determinations made, as 0.007μ per second. This rate was reduced in a moderately old internode to 0.0017μ per second, which is one-fourth that in a young internode. In a still older internode the rate is reduced to about one-tenth, or may even become arrested.

Experiment 3. *Effect of light on a moderately old internode of Cajanus.*—The rate of diametric growth of the internode was feeble, being 0.0017μ per second. In the record of normal growth N, the ascending curve therefore shows only a slight inclination (fig. 52). After securing the balance, the record B is horizontal. On application of light at arrow, there is an upsetting of the balance in an *upward* direction, indicating an acceleration of the rate of growth. This acceleration persists for a certain length of time, even after the withdrawal of light at arrow within a circle. The original rate is then restored as indicated by the resumption of horizontality of the balanced curve.

¹ Bose, *Growth and Tropic Movements of Plants* (1929), p. 91.

Experiment 4. *Effect of light on growth of a still older internode.*—The rate of diametric growth was so feeble, that the normal curve N of growth was nearly horizontal, and

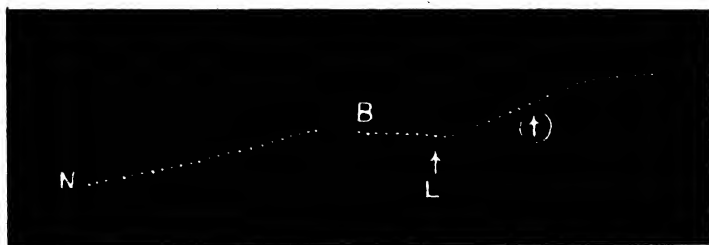


FIG. 52. Effect of light on diametric growth of a moderately old internode of *Cajanus*.

Light induces an *acceleration* of the rate of growth.

it was therefore unnecessary to secure balance of growth. Light was applied at arrow with the result of an erection of the curve, indicative of an enhancement of the rate of diametric growth. This enhancement persisted for a con-



FIG. 53. Effect of light on diametric growth of an older internode of *Cajanus*.

Acceleration of the rate as in previous case.

siderable length of time, even after the withdrawal of light at arrow within a circle (fig. 53).

In summarising the results of the effect of light on diametric growth, it may be stated that *while photic stimulus induces a retardation of the rate of vigorously growing young internodes, it causes an ACCELERATION in old internodes in*

which the growth had become enfeebled. Light thus induces two opposite effects depending on the age of the organ.

An actively growing organ may conveniently be distinguished as in a favourable tonic condition, while that exhibiting enfeebled growth may be regarded as in a condition of subtonicity. It may be said in general, that this subtonicity is brought about, not only by unfavourable conditions of the environment, but also by the age of the organ. A large number of experiments carried out on the subject lead to the conclusion that the growth of a subtonic organ is enhanced or revived by the stimulus of light.

Further experiments will next be described which tend to show that the normal retardation of growth by light is due to its functioning as a stimulus in inducing an excitatory reaction.

EFFECT OF ELECTRIC STIMULUS ON DIAMETRIC GROWTH

It has been stated that the excitatory impulse generated by the incidence of the stimulus of light percolates inwards and, reaching the responding layers of growth, gives rise to an incipient contraction and retardation of the rate of growth. The assumption that light functions as a stimulus will receive full confirmation if it can be shown that an electric stimulus which is known to induce excitation causes reactions essentially similar to those under light.

METHOD OF EXPERIMENT

Electric stimulation is produced by an induction coil, usually employed for physiological investigations. By keeping the primary coil at fixed distance from the secondary, and by application of the stimulus for a definite duration, the effective intensity of the electric stimulus can be maintained constant in all the different experiments. The duration of the shock is maintained constant by the interposition of a metronome in the primary circuit of the induction coil.

Experiment 5. *Effect of electric stimulus on diametric*

growth of Cajanus.—The record of normal diametric growth N is first taken, the rate in the present case being 0.008μ per second. The balanced record B is horizontal; on application of electric stimulus S at the down-pointing arrow, there is produced a sudden upsetting of the balance downwards indicating a pronounced retardation of the rate of growth. It is to be noted that the successive dots in the down-curve exhibit comparatively wide intervals, which demonstrate a very rapid contractile response in retardation of growth. After the attainment of maximum contraction, the horizontal portion of the record shows a recovery to the

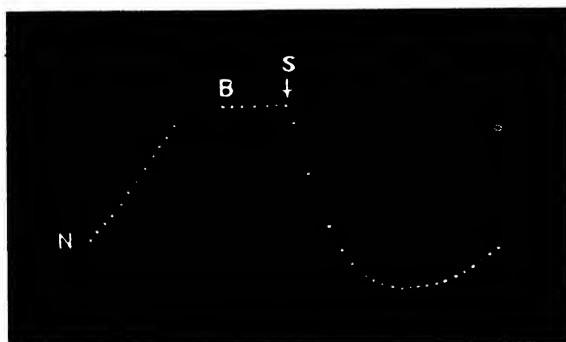


FIG. 54. Effect of electric stimulus *s* on balanced diametric growth of thin stem of *Cajanus*.

original rate of growth. After a time the curve exhibits even a slight ascent, proving that the after-effect of electric stimulus is similar to that of light, namely, a short-lived enhancement of growth above normal (fig. 54). As regards the effect on thin stems, the action of electric stimulus is very similar to that of light. Does the similarity extend also in the case of thick stems?

Experiment 6. *Effect of electric stimulus on diametric growth of thick stem of Antirrhinum.*—The first part of the record N exhibits the rate of normal diametric growth, which is found to be 0.010μ per second. B is the horizontal record of balanced growth; application of electric stimulus S at the down-pointing arrow is seen to cause an abrupt upsetting of the balance *downwards*, proving that the induced

retardation of growth is as marked as in the previous case. The after-effect of the stimulus is also essentially similar in the two cases, *i.e.* a short-lived enhancement of the rate of diametric growth above the normal (fig. 55).

It will thus be seen that under electric stimulation there is no difference between the response of thin and thick stems, the final reaction in each occurring almost instantaneously. A fuller explanation of this will be presently given.

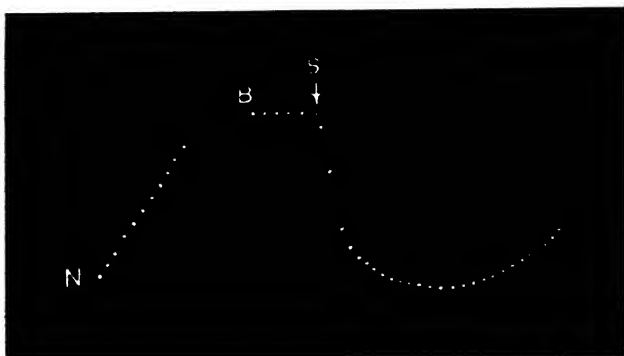


FIG. 55. Effect of electric stimulus *s* on the balanced diametric growth of thick stem of *Antirrhimum*.

The tabular statement on p. 99 of typical results of the effect of electric stimulation proves the essential similarity between the responses of the thin and thick stems of the two different species of plants.

Certain characteristics of the effects of light and of electric stimulus are of special interest since, in spite of apparent differences, they offer independent evidence in support of the essential similarity of different modes of stimulation in modification of growth. The effects induced by light and by electric stimulation on the two species of plants are summarised below :

- (1) The thin stem of *Cajanus* under the action of light exhibits a retardation of rate of growth or a negative response.

- (2) The thick stem of *Antirrhinum* under the action of light exhibits a preliminary acceleration followed by a retardation of the rate of growth, that is to say, a preliminary positive followed by a negative response.
- (3) Under the action of electric stimulus, however, both *Cajanus* and *Antirrhinum* show an immediate retardation in the rate of growth or a negative response.

TABLE III.—EFFECT OF ELECTRIC STIMULUS ON DIAMETRIC GROWTH OF *CAJANUS* AND *ANTIRRHINUM*

Specimen	Rate of diametric growth per second	Immediate effect of stimulus	Transient after-effect
1. <i>Cajanus</i>	0.007 μ	Retardation	Acceleration
2.	0.008 μ	„	„
3. <i>Antirrhinum</i>	0.008 μ	„	„
	0.009 μ	„	„
5.	0.009 μ	„	„
6.	0.010 μ	„	„

The variations of response referred to above find full explanation from the following consideration. In regard to the difference of reaction in thin and in thick stems under light, it has already been explained that the excitation generated by photic stimulus, incident on the surface of the

stem, gradually percolates through the intervening tissues, and, reaching the growth-layer, induces an incipient contraction and retardation of the rate of growth ; the interval between the stimulus and responsive reaction is therefore determined by the thickness of the intervening tissue. The difference of thickness of stem in the two species of plants, as already explained, accounts for the response in one case being negative while in the other the negative is preceded by a preliminary positive. Turning next to the normal negative response under photic stimulus, since the excitation passes slowly across the successive layers of growth the total retardation becomes gradually summated. The short intervals between successive dots in the recorded down-curve represent the slowness of this increasing retardation (*cf.* fig. 50). But in regard to the action of electric stimulus, no delay intervenes between the contractile responses of successive layers of growth, since the stimulus instantly passes through all the tissue, both external and internal. Stimulation being direct, the excitatory contraction and retardation of growth occur simultaneously throughout all growing layers. In the record itself the relative rapidity of the total reaction is exhibited by the wide spacings between the successive dots of the down-curve, which represents contraction and abrupt diminution of the rate of diametric growth (*cf.* fig. 55). In other respects the induced reactions are essentially similar under photic and electric stimulations. The after-effects of both modes of stimulation are also similar, being a transient enhancement of the rate of growth above the normal (*cf.* figs. 50, 54, 55).

The results obtained prove :

- (1) That there is a retardation of diametric growth under the action of light, the excitatory impulse generated being transmitted inwards.
- (2) That the interval between the impact of stimulus of light and the induced variation of the growth depends on the thickness of the tissue which intervenes between the surface and the responding layer of growth.

- (3) That when the intervening thickness is considerable, as in the thick stem of *Antirrhinum*, the stimulation is indirect, the characteristic effect of which is an acceleration of the rate of growth ; the transmitted excitation, however, reaches the layer of growth later and induces the normal retardation of the rate. The complete responsive formula is therefore a preliminary positive acceleration followed by a negative retardation.
- (4) That the effect of electric stimulation in retardation of growth is essentially similar to that of the photic stimulation. This retardation is, however, immediate and abrupt on account of the instantaneous passage of electric shock throughout all layers of tissues, both external and internal.

EFFECT OF DIRECT AND OF INDIRECT STIMULATION ON DIAMETRIC GROWTH

The opposite effects of direct and indirect photic stimulation on growth have already been referred to (*cf.* Experiment 2). In order to demonstrate the universality of the phenomenon, the following investigation was undertaken on the characteristic effects of direct and indirect electric stimulation on diametric growth of stems.

Experiment 7. *Effect of direct electric stimulation on Cajanus.* An experiment on this subject has already been described (*cf.* Experiment 5). In order to ensure an identical climatic condition in observation of the effects of direct and indirect stimulations, the two experiments were completed with two typical specimens in the course of the same day. In fig. 56 is shown the effect of direct stimulation on balanced growth, the result being a pronounced retardation of the rate of growth as indicated by the down-curve in the record.

Experiment 8. *Effect of indirect electric stimulation on Cajanus.*—The indirect stimulus of electric induction shocks of moderate intensity was applied on the other specimen at a distance of 1 cm. below the active region of growth. After

taking the record N without balance, the balanced horizontal record B is obtained. The application of indirect electric stimulation I at the up-pointing curve is seen to produce an

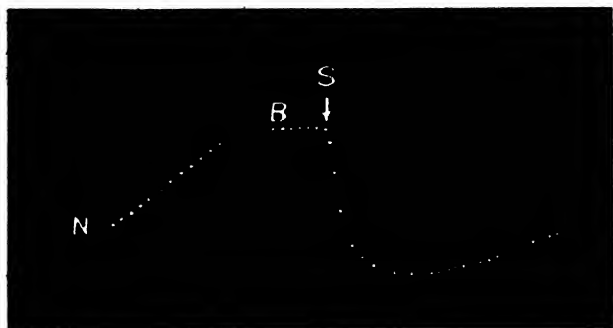


FIG. 56. Effect of direct electric stimulation *s* on the balanced diametric growth of *Cajanus*, exhibiting very rapid contraction and retardation of rate of growth.

upsetting of the balance *upwards*, indicating an enhancement of the rate of growth above the normal (fig. 57).

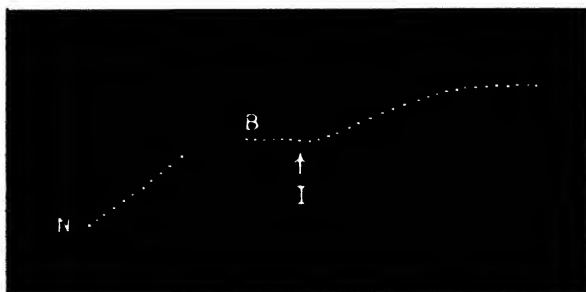


FIG. 57. Effect of indirect electric stimulation *i* on balanced diametric growth of *Cajanus*.

The response to indirect stimulation is of opposite sign to that of direct stimulation, being an acceleration of the rate of growth.

The experiments that have been described prove that *the effect of a stimulus is modified by its point of application ; direct stimulation induces a retardation, while indirect stimulation brings about an acceleration of the rate of growth.*

EFFECT OF A CONSTANT ELECTRIC CURRENT IN ASCENDING AND DESCENDING DIRECTIONS ON DIAMETRIC GROWTH

The effects of induction shocks on growth have been given above. The next investigation is undertaken to find whether the passage of a constant electric current induces any variation in the rate of growth. In order to find out whether a constant current has any effect on growth according to its direction, two successive experiments were carried out with an identical specimen under the action of an ascending and a descending current. By the *ascending* is meant a current which flows in the stem from the old internode below to the young internode above, while the *descending* indicates a current which flows in the opposite direction, from the young internode above to the old internode below.

The idea underlying this investigation was that, since growth is enhanced by an increase of turgor and depressed by its diminution, the directive action of an electrical current might indirectly cause an acceleration or retardation of the rate of growth. This can only be true if an electric current modified the ascent of sap to such a marked degree that the current in one direction increased the rate of rise of sap with concomitant increase of turgor and enhancement of the rate of growth, while the current in the opposite direction, contrariwise, retarded or arrested the rise of sap and caused a diminution of turgor and retarded the rate of growth.

The effects of ascending and descending currents on growth will now be described and an attempt will be made to offer an explanation of the observed phenomena.

METHOD OF EXPERIMENT

The record of diametric growth of a young internode of a stem is taken in the usual manner. The constant current from a battery of storage cells, having an E.M.F. of about ten volts, is sent in succession, either in an ascending or in a descending direction, the change of the direction of the current being effected by means of a Reversing Key. Suitable electric connections are made with the stem for

passage of the current. It is to be remembered that the record of growth and its induced variation are taken at the actively growing region in the young internode. In regard to the two electric connections, the upper is made at the point in the young internode, slightly above the section of which the growth is being recorded; the lower connection, on the other hand, is made at the old internode in which growth is practically at a standstill. The current is ascending when the Anode or the point of entry of the current is below, while the Kathode or the point of exit of the current is above. The current is descending when the Anodic point is above and the Kathodic point below.

Experiments were carried out with a large number of the two species of plants, *Cajanus* and *Antirrhinum*, the results obtained being found to be essentially similar. Detailed accounts of three typical experiments are given below.

Experiment 9. *Effect of ascending and descending currents on diametric growth of Cajanus.*—The normal rate of diametric growth was 0.009μ per second. After taking the record of normal growth N, the balanced record B was taken. In this and in all the following experiments the current employed was from a ten volts battery, its intensity being approximately 84 micro-amps. On starting the ascending current at the up-pointing arrow *a*, the result was *an upsetting of the balance in a downwards direction, indicative of a retardation of the rate of growth. This induced retardation persisted only for a relatively short time, after which the record became once more horizontal, indicating a restoration to the original rate of growth.* Though the constant current was kept on for nearly two minutes, no further change was noticed. In the experiment just described, the Kathodic point K was above, the Anodic point A below. The current was next reversed at the down-pointing arrow *d*, resulting in a descending current, the Anode being now above and the Kathode below. *Inspection of the record shows that no change was induced since the curve remained horizontal (fig. 58). This proves that the descending current has practically no effect in inducing any variation in the rate of growth.*

Experiment 10. *Effect of descending and ascending currents on diametric growth of Cajanus.*—In this experiment

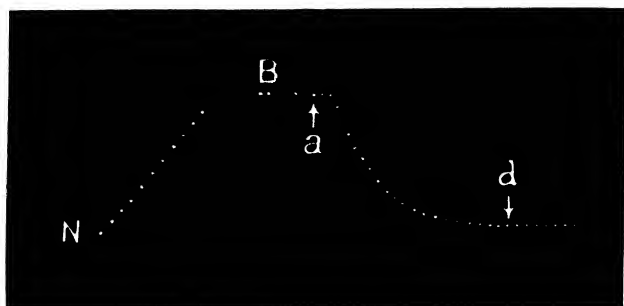


FIG. 58. Effect of ascending current *a* and descending current *d* on the diametric growth of *Cajanus*.

The ascending current *a* induces a transient retardation of the rate of growth, the descending current *d* being without any effect.

the sequence of application of the two currents was changed, the effect of the descending current being recorded before that of the ascending current. The rate of the diametric

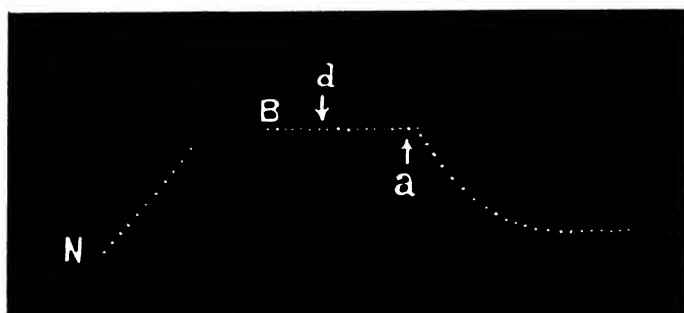


FIG. 59. Effect of descending current *d* and of ascending current *a* on diametric growth of *Cajanus*.

In spite of the sequence of ascending and descending currents being changed, the results are similar to that given in fig. 58.

growth of the specimen was 0.0081μ per second. The normal record of growth is seen at N, and the balanced record at B. The descending current was applied at the down-pointing arrow *d*, and maintained for nearly two minutes. The record (fig. 59) shows that the descending

current had induced no variation in the rate of growth, as indicated by the record remaining horizontal. But when, by reversing the direction of the current at the up-pointing arrow *a*, it was made ascending, there was a short-lived retardation of the rate of growth, after which the record became once more horizontal.

Experiment II. *Effect of ascending and descending currents on diametric growth of Antirrhinum.*—The method of procedure was similar to that employed with *Cajanus*. The rate of diametric growth of the specimen was 0.011μ per second, indicating a growth-activity relatively greater than in *Cajanus*. The first part of fig. 60 indicates the normal

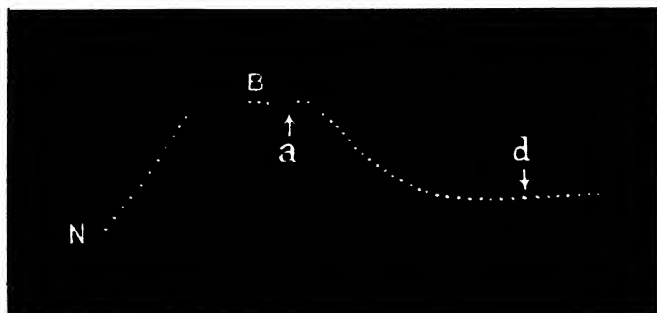


FIG. 60. Effect of ascending current *a* and of descending current *d* on the diametric growth of *Antirrhinum*.

The effects are essentially similar to those in *Cajanus*.

record of growth *N*. After obtaining the balanced horizontal record *B*, an ascending current *a* was started at the up-pointing arrow. The ascending current caused, after a short latent period, a pronounced upsetting of the balance downwards, indicative of a marked retardation of the rate of growth. This retardation, as in *Cajanus*, was short-lived, for the record became once more horizontal. When the current was reversed, the descending current *d* did not induce any change in the rate.

The stems of two different species of plants thus exhibit reactions which are essentially similar. The following table gives a summary of typical results obtained with five different specimens.

TABLE IV.—EFFECTS OF CONTINUED ACTION OF ASCENDING AND DESCENDING CURRENTS ON DIAMETRIC GROWTH

Specimen	Rate of growth per second	Effect of ascending current on growth	Effect of descending current on growth
1. <i>Antirrhinum</i>	0.011 μ	Transient retardation	No change
2. <i>Cajanus</i>	0.008 μ	as above	as above
3. „	0.008 μ	as above	as above
4. „	0.009 μ	as above	as above
5. „	0.009 μ	as above	as above

The results described above prove that the induced change of growth could not have been due to any modification of the rate of ascent of sap under the directive action of the electric current. If this had been the case, then the retardation of growth induced by the current flowing in one direction should have persisted throughout the duration of the current. The effect actually observed was, however, of a transitory nature. Again, the effect of reversing the direction of the current should have brought about an acceleration of the rate of growth; this reversal of the current did not, however, cause any change in the rate of growth.

It is, therefore, clear that the modification of the rate of growth under the action of a constant current could not be due to its directive action as such, but to some other effect of the electrical current. For obtaining a full and satisfactory explanation of the observed effects it would be necessary to make a short reference to the polar action of a constant current.

POLAR ACTION OF A CONSTANT CURRENT IN
EXCITATION

It has been shown that any agent which induces an excitatory reaction brings about a retardation of the rate of growth. This conclusion was demonstrated by the action of the stimulus of light as well as that of electric shocks from an induction coil. It may now be asked, whether a constant electric current causes any excitatory reaction, and if so, what are its characteristics?

With regard to special investigations on this subject, Sir J. C. Bose has described elsewhere his discovery of the polar action of a constant electric current in excitation. He found that the reactions induced by it are essentially similar in the animal and in the plant tissues. Thus the effects of a moderately feeble constant current are (1) a short-lived excitatory reaction at the Kathode on the sudden starting or 'make' of the current; (2) that the excitation does not persist during the continuation of the current; and (3) that the 'make' of the Anode does not induce any such excitatory reaction.¹

An attempt will now be made to find whether the above discovery of the polar action of current in excitation of plant tissues offers a satisfactory explanation of the characteristic effects on growth, which have been observed under the action of ascending and descending currents and described in the foregoing experiments. It will be remembered that the electric connections for the particular investigation are as follows: the upper connection is made very near the active region of growth in the young internode situated above, while the lower connection is made with the old and non-growing internode below. It therefore follows that when the current is ascending it is the Kathode which is near the region of growth in the young internode, the induced variation of which is to be recorded. The sudden 'make' of the ascending current, therefore, is equivalent to Kathode-make, the excitatory effect of which would induce a *transient* retardation of the normal rate of growth. This is the characteristic effect that was observed under the action of

¹ Bose, *The Nervous Mechanism of Plants* (1926), p. 26.

an ascending current, namely, a transient retardation of the rate of growth as demonstrated by the records given in figs. 58, 59 and 60.

Next, when the current is reversed or descending, the Anode takes the place of the Kathode in the active region of growth of the young internode situated above. On starting this descending current the active region of growth is subjected to the action of Anode-make which, as already stated, has no excitatory reaction. Hence the descending current cannot induce any retardation in the rate of growth. This has actually been demonstrated by series of records given in figs. 58, 59, 60, which show that no such variation is induced in the rate of diametric growth by the action of a descending current.

The observed effects of ascending and descending currents are therefore due, not to the directive action of the current as such, but to the discriminative polar action of a current in excitation.

PHOTOTROPIC CURVATURE UNDER THE ACTION OF UNILATERAL LIGHT

The investigations on the effect of light that have been described relate to its simultaneous action on all sides of the organ. The following experiments are on the action of unilateral light in inducing the characteristic tropic curvature. The induced responsive movement was recorded by a single recording lever, which produced a magnification of 100 times; the record was taken on an oscillating smoked plate of glass, the frequency of oscillation being once in 15 seconds. In order to prevent overlapping, two of the intermediate dots have been omitted, and in the records reproduced, the spacing between successive dots therefore represents a time-interval of 45 seconds.

One of the special objects of the present inquiry is to find whether the tropic reaction is in any way modified by the age of the internode on which the light is made to act. The experiments were therefore carried out on young, on old, and on intermediate internodes of the stem of *Cajanus*.

Experiment 12. *Phototropic effect of unilateral light on a young internode of Cajanus.*—The record (fig. 61) shows that, on the application of unilateral light, at the up-pointing arrow, the stem exhibited a bending towards it. This bending towards the stimulus will be distinguished as a positive phototropic movement and is represented in the record by a down-curve. This positive movement was initiated in the course of 1.5 minutes and persisted, even after the removal of light at the horizontal arrow within a circle, for more than 7 minutes. There was afterwards a recovery which was more or less complete.

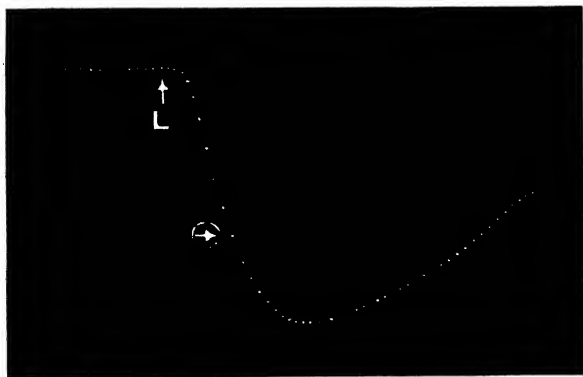


FIG. 61. Phototropic effect of unilateral light on a young internode of *Cajanus*.

Positive response towards the light represented by the down-curve. Interval between successive dots in this and in following records is 45 seconds.

Experiment 13. *Phototropic effect of unilateral light on a moderately old internode of Cajanus.*—In this case the movement of response initiated after a longer latent period was negative or away from the light, represented by an up-curve. The negative movement persisted for a considerable length of time, after which there was a partial recovery (fig. 62).

It is thus seen that the induced phototropic curvature is modified by the age of the internode, the sign of response of a moderately old being precisely opposite to that of a young internode. The phototropic response is therefore dependent on the age of the organ.

How are these opposite effects to be explained? It has been shown, that in regard to the action of the stimulus of light, incident on all sides of the young internode, there is induced a retardation of the rate of diametric growth (*cf.* Experiment 1). When light acts only on one side that side alone undergoes incipient contraction, and exhibits retardation of the rate of growth; the distal side, which is protected from light, may, on the other hand, continue its normal growth.

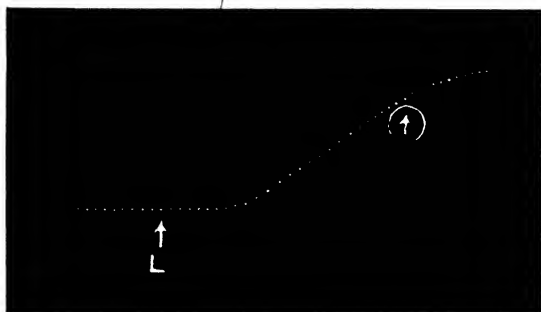


FIG. 62. Negative phototropic effect of unilateral light exhibited by an old internode of *Cajanus*.

It should, however, be borne in mind that the indirect stimulation of the distal side is likely to cause an enhancement of the rate of growth of that side. The differential effects induced by the stimulus on the two sides, therefore, bring about the concavity of the proximal and the convexity of the distal side, which result in a positive curvature towards the light. In addition to this one-sided modification of the diametric growth, a variation of longitudinal growth also comes into operation. For the growth in length of the stem is retarded at the stimulated proximal side, while the unstimulated distal side exhibits no retardation, if not actual acceleration of the normal rate. The effects of both these factors, namely, induced variation of longitudinal and of diametric growth, conspire to induce the observed positive curvature.

In regard to the negative phototropism, exhibited by an old internode, reference may be made to Experiments 4 and

5, which show that simultaneous stimulation of all sides of the organ by light induces, in an old internode, an *acceleration* of the rate of diametric growth instead of the normal retardation. Unilateral light will, therefore, enhance the diametric growth of the proximal side which alone is stimulated by light, thus giving rise to convexity of that side and the resulting negative phototropic curvature.

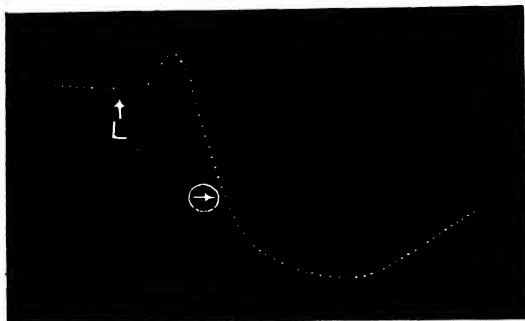


FIG. 63. Negative followed by positive phototropic response exhibited by an intermediate internode of *Cajanus*.

Experiment 14. *Phototropic curvature of an intermediate internode.*—The results given in Experiments 12 and 13 relate to the responsive phototropic movements of a very young and of a moderately old internode. There remains the transitional case in which the internode is neither too young nor too old. Here arises an intermediate case, in which the preliminary response is negative or away from light, followed by a positive movement towards the light (fig. 63).

When the entire length of one side of the stem is exposed to unilateral light, the effect becomes far more complicated. For the resulting response of the apex of the stem is then determined by the algebraical summation of two different movements, positive and negative, which occur in the young and in the old internode. The above facts afford a satisfactory explanation of the highly complex phototropic response often exhibited by stems.

SUMMARY

The *Diametric Growth Recorder* has been specially devised for obtaining automatic record, by which the absolute rate of diametric growth and its induced variation can be determined with the highest accuracy. The sensitiveness of the device has further been greatly augmented by the Method of Balance.

The effect of light on diametric growth in thin stems of young internodes of *Cajanus* is a retardation of the rate of growth. But in thicker stems of *Antirrhinum* the effect of light is a preliminary acceleration followed by normal retardation in the rate of growth.

An explanation of this difference is found in the fact that while direct stimulation induces a retardation, indirect stimulation brings about an acceleration in the rate of growth. In thick stems it takes a considerable length of time for the effect of superficial stimulation by light to percolate through the intervening depth of the tissue, on account of which the stimulation at the beginning is indirect.

The modification of growth induced by light is due to its excitatory action. This is proved by the fact that a non-photic electric mode of stimulation, which is effective in causing excitation, induces parallel reactions.

Under electric stimulation there is no difference in the effects induced in thin and in thick stems. This is due to the fact that the electric shock passes instantaneously through all tissues, both external and internal; the stimulation is therefore direct and causes the normal retardation of the rate of growth.

The polar effect of the 'make' of a constant current is an excitatory reaction of contraction, at or near the point of Kathode, giving rise to the retardation in the rate of growth in the actively growing region. This result is transitory, since the excitatory effect of the sudden 'make' of the Kathode is short-lived; hence the induced retardation of growth does not persist during the continuation of the current. When the direction of the current is reversed, the Anode takes the place of the Kathode and there is no excitatory effect in retardation of growth by the 'make' of the Anode.

This explains the effects of 'ascending' and of 'descending' currents, the characteristic reactions being due not to the directive action of a current as such, but to the discriminating polar action of a constant electric current in inducing excitation, which results in the retardation of the rate of growth.

The effect of light on diametric growth is modified by the age of the specimen. It is shown, that while photic stimulus induces a *retardation* of growth in a vigorously growing young internode, it causes an *acceleration* of the rate in an old internode in which growth has undergone a decline. Light thus induces two opposite effects, which depend on the age of the organ.

The tropic effect on the stem under unilateral action of light is found to be modified under definite conditions as a positive movement towards the light, or a negative movement away from it. A very important factor in determining the sign of reaction is the differential effect of light on the proximal and distal sides of the organ. When light is incident unilaterally on a vigorously growing internode, there is a retardation of the rate of diametric growth on the proximal side due to direct stimulation. The indirect effect on the distal or shaded side is, on the other hand, an acceleration of the rate of growth. The induced concavity of the proximal and the convexity of the distal side thus result in the *positive* phototropic curvature.

But when the light is incident on a moderately old internode, the responsive movement is negative or away from light. This is explained by the fact that light induces in such old organs an *acceleration* of the rate of diametric growth at the directly stimulated side. Under unilateral light the proximal side thus becomes convex, resulting in a *negative* phototropic curvature.

When the entire length of one side of the stem is exposed to unilateral light, the effect becomes far more complicated. For the resulting movement of the apex of the stem is then determined by the algebraical summation of two different movements, positive and negative, which occur in younger and in older internodes.

VI.—THE AUTOMATIC RECORD OF GROWTH OF ROOTS AND ITS INDUCED VARIATION

BY

A. GUHA-THAKURTA, C.S.AG.C., AND B. K. DUTT, B.Sc.

THE exact determination of the normal rate of growth of roots and its induced variation under the action of external agents are problems attended by exceptional difficulties. The only attempt in this direction has been by taking frequent readings of the micrometric scale in the eye-piece of a microscope focussed on the tip of the root. The method of eye-observation, subject as it is to personal errors, cannot give very accurate results, nor does it allow the detection and exact determination of the *preliminary* in contrast with the *persistent* variation in the rate of growth induced by changes of external agencies.

The only means of satisfactorily solving the problem is the invention and successful construction of a device by which the growth of the root, and the variation induced in it, can be instantly and automatically recorded. The period of the experiment should be greatly shortened in order to avoid the complications which might possibly arise from the effect of changes in the environment, such as temperature and light. This necessitates the obtaining of the record at a very high magnification, so that the normal rate of growth and its induced variations can be quickly determined.

These desiderata have been fulfilled by the *High Magnification Crescograph*, described elsewhere, by which the growth-elongation of stems is recorded.¹ In this contrivance the tip of the stem is connected by a thread to the short arm of the first lever, while its long arm is attached to the short arm of

¹ Bose, *Growth and Tropic Movements of Plants*, p. 11.

the second recording lever, the compound magnification thus produced being from 4000 to 10,000 times. The curve of growth is taken on an oscillating plate, moving to and fro at definite intervals of time; in the dotted record thus produced, the distance between the successive dots represents the magnified growth which occurs during a definite short interval of time.

Exceptional difficulties are, however, encountered in the employment of this contrivance for the automatic record of growth of roots. In the case of a stem it is not difficult to attach its upper end to the first of the system of magnifying levers; but in the case of a root, its slender tip cannot be attached in the same way. An additional difficulty which is encountered in recording the growth of roots arises from the fact that while the movement of growth in the stem is upwards, that of a root is downwards; this drawback cannot, however, be overcome by holding the root upside down, for then its normal rate of growth would be affected by geotropic action. The difficulties were ultimately overcome by obtaining a highly magnified record of the *downward pressure exerted by the growing tip of the root*.

The following experiments were carried out not only with aerial roots, but also with roots which grow underground. For the former we employed the aerial roots of *Vitis quadrangularis*, and for the latter the roots of *Pandanus odoratissimus*. A necessary condition for the success of the investigation is that the principal or the lateral root employed for the experiment should be sufficiently stiff for exerting an effective downward pressure, the magnified record of which would enable the determination of the rate of growth and its induced variation.

The results of investigations will be described in the following order :

- (1) Effect of different gases on the growth of aerial root.
- (2) Effect of different gases on the growth of underground root.
- (3) Effect of electric stimulus on the growth of root.
- (4) Effect of stimulus of light on the growth of root.

METHOD OF EXPERIMENT

A detailed description of the method of recording the growth of aerial root will explain the general principle involved. Since the employment of the whole plant would be highly inconvenient for necessary manipulations, a fairly

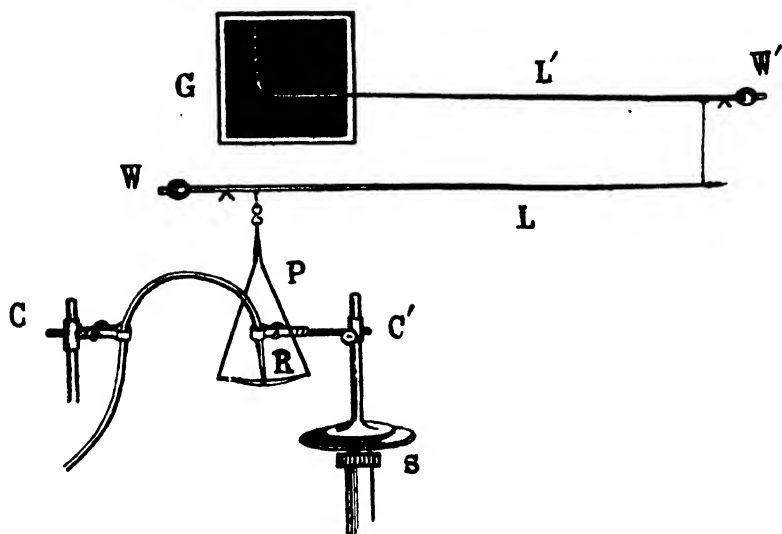


FIG. 64. High Magnification Automatic Recorder for growth of roots.

Detached aerial root suitably held by clamps *c* and *c'*. Active region of growth *R* is held from above by clamp *c'* which can be adjusted up or down by micrometer screw *s*. Tip of root presses against a suspended aluminium pan *P*, attached at a short distance from fulcrum of first lever *L*. The recording lever *L'* records growth-elongation on oscillating smoked glass plate *G*. *w* and *w'*, counterpoises for approximate balancing of long arm of levers.

long root of *Vitis quadrangularis* is detached from the parent plant. The irritation caused by this operation brings about a depression or arrest of growth; the normal rate is, however, restored after a period of rest, the root having then resumed its normal rate of growth. The detached root is now suitably held by the clamps *C* and *C'* (fig. 64). The root above the region of active growth *R*, held by the second

clamp C', can be very gradually raised or lowered by means of the adjusting micrometer screw S. By this special adjustment the tip of the root can be made to press slightly against a suspended aluminium pan P, which is attached to the first lever L at a short distance from the fulcrum. This first lever is suitably attached to the second or recording lever L'. Each of the two levers is nearly balanced by suitable counterpoises W, W'. The growth-elongation of the tip of the root exerts a pressure on the pan which pulls down the first lever. This downward movement of the first lever, caused by growth-elongation, is further magnified by the recording lever. The first lever magnifies 50 times and the second lever 80 times, the compound magnification being therefore 4,000 times. The actual record is obtained on a smoked glass plate, which is kept oscillating to and fro, say, at intervals of 10 seconds, producing a dotted curve; the distance between successive dots therefore represents magnified growth-elongation in the course of 10 seconds. In the case of very slow-growing roots the time-interval between successive dots is increased to 40 seconds, so that the dots are sufficiently apart and not superposed on each other.

Precautions to be taken.—These relate (a) to the reduction of the curvature in the record; (b) to the maintenance of fairly constant pressure of the root on the pan during the course of each record; and (c) to the obtaining of successive series of record of growth on the smoked glass plate.

The length of the recording lever is 20 cm. As the tip of the lever moves in an arc, a curvature is naturally produced in the record. The use of the middle portion of the growth-record reduces the curvature to a minimum.

As regards the tension of the system of levers against which the root has to exert pressure, this remains approximately constant during the excursion of the recording lever from a position slightly above to a position slightly below the horizontal line. The necessary adjustment of the position of the recording lever is made by means of the micrometer screw S. Two different methods are employed for securing the record; in one of these the plate is maintained stationary, while in the other it is kept moving in a lateral direction.

In the *Stationary Plate Method* the smoked glass plate is kept in a particular place and a dotted record of growth obtained on the oscillating plate. After the completion of the first series, the plate is pushed laterally through about 1 cm. and left there for the subsequent series. The recording lever is suitably readjusted for each series of record by means of the micrometer screw already referred to. Comparison of the successive series of records enables the determination of the uniformity of the growth-rate or the variation induced in that rate by external agencies.

In the second or *Moving Plate Method* the smoked glass plate moves laterally at uniform rate by means of clockwork.

The curve of growth-elongation, obtained according to the particular arrangement of the compound lever, is downwards. As the elongation is usually represented by an up-movement, the record reproduced is therefore printed inverted so that the growth elongation appears as an up-curve.

The absolute rate of growth per second is found from the magnification employed and the time-interval between successive dots in the record. The enhancement or retardation of the rate of growth under a particular agent is visually demonstrated by the increase or the diminution of the distance between the successive dots.

DETERMINATION OF THE ABSOLUTE RATE OF GROWTH

In the determination of the absolute rate of growth a second is adopted as the unit of time, and μ or micron as the unit of length. The micron, as previously stated, is a millionth part of a metre or a thousandth part of a millimetre.

If m be the magnifying power of the compound lever, l the average distance between the successive dots in mm., and t the time-interval between successive dots in seconds, then :

$$\text{the rate of growth} = \frac{l}{mt} \times 10^3 \mu \text{ per second.}$$

Experiment 1. *The uniformity of the rate of normal growth of the root of V. quadrangularis.*—When the external

condition is kept constant, then the rate of growth is found to be very uniform. This is demonstrated by the three series of the records *a*, *b*, *c*, in fig. 65, in which the successive dots are at intervals of 10 seconds. After taking the first series *a*, the series *b* and *c* were taken each half an hour after the preceding series. The recorded lengths which occur during 12 spacings, representing 12×10 seconds or 2 minutes, are seen to be equal. This proves that the rate

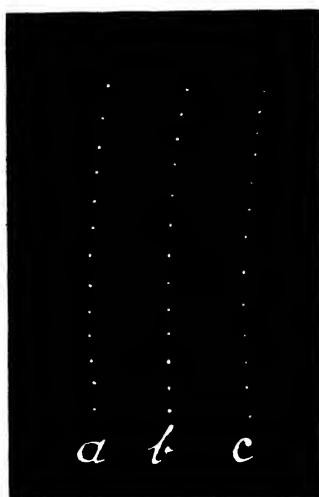


FIG. 65. Recorded growth of root of *Vitis quadrangularis*.
The second and third records were obtained half an hour and one hour after the first.

Successive dots at intervals of 10 seconds.

of growth under constant external conditions remains uniform for at least an hour.

Since the growth-elongation in the course of 120 seconds is 43 mm. under a magnification of 4000 times :

$$\begin{aligned} \text{the absolute rate of growth} &= \frac{43 \times 10^3}{4000 \times 120} \mu \text{ per second.} \\ &= 0.09 \mu \text{ per second.} \end{aligned}$$

In the particular specimen of root the rate of growth is 0.09μ per second ; but in others it may vary between

0.06 μ to 0.18 μ per second. In contrast with the rate of growth of roots that of stems is considerably higher, being of the order of 0.5 μ per second. One of the causes for the comparatively slow rate of growth-elongation of the root may perhaps be due to the fact that the actively growing length of the root is shorter than that in the stem.

EFFECT OF DIFFERENT GASES AND VAPOURS ON GROWTH OF AERIAL ROOTS

The modification of growth of aerial roots under the action of different gases and vapours will now be described. The results of investigations on the effect of various agencies on the growth of underground roots will be given later. For the purpose of this particular investigation the actively growing root is surrounded by a light mica cylinder for the application of different gases and vapours. A particular gas is introduced into the cylindrical chamber, and the effect either of a short or of a prolonged application is then recorded. The record obtained on removal of the gas and substitution of fresh air enables the determination of the after-effect of application.

Two different methods, as previously indicated, are employed in the following investigations. The result obtained by the first or the Stationary Plate Method is in some cases independently verified by the second or the Moving Plate Method. The sensitiveness of the latter is greatly enhanced by the further employment of the Method of Balance, which offers a unique opportunity of discovering the characteristics of different phases of reaction, namely, the preliminary, the permanent and the after effects of the application of different agents.

EFFECT OF APPLICATION OF CO₂ ON AERIAL ROOT OF *VITIS QUADRANGULARIS*

STATIONARY PLATE METHOD

Experiment 2. *Effect of dilute CO₂ on growth of aerial root.*—The first record *a* of the series (fig. 66) shows the

normal rate of growth, which was 0.07μ per second. The immediate responsive variation which occurred in the course of two minutes was a very great enhancement of the rate of growth, which was nearly three times the normal rate as shown in the record *b*. The enhancement persisted even after continued application for 5 minutes (record *c*). Continual application for a period of ten minutes induced

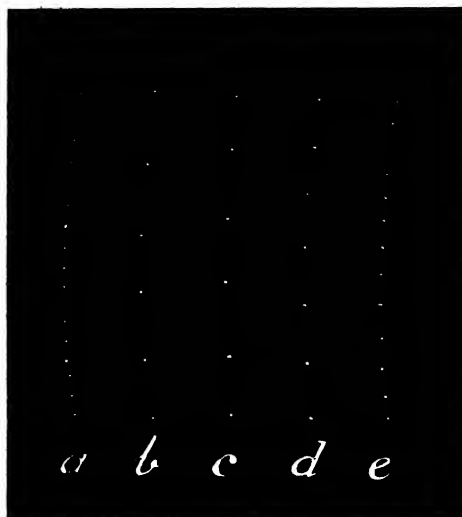


FIG. 66. Effect of dilute CO_2 on growth of the root of *Vitis quadrangularis*.

a. Record of normal rate.

b, c, d, e. Records of effects of increasing duration of application.

only a slight diminution of the enhanced rate (record *d*). Even after the prolonged application of dilute CO_2 for an hour there is no actual depression (record *e*). In fact the rate now was 0.09μ per second, being still greater than the normal rate of 0.07μ per second.

The detailed results are given in the tabular statement on p. 123.

It will be seen from this that the effect of dilute CO_2 is an acceleration of the rate of growth, which persists for a considerable length of time.

TABLE I.—EFFECT OF APPLICATION OF DILUTE CO_2 ON GROWTH OF AERIAL ROOT OF *VITIS QUADRANGULARIS*

Normal rate	0.07 μ per second
After application for 2 minutes	0.20 μ „ „
„ „ „ 5 „	0.20 μ „ „
„ „ „ 10 „	0.18 μ „ „
„ „ „ 60 „	0.09 μ „ „

Experiment 3. *Effect of strong CO_2 .*—A more concentrated carbonic acid gas was next applied. In the series of records given in fig. 67 are shown: *a*, the normal rate of

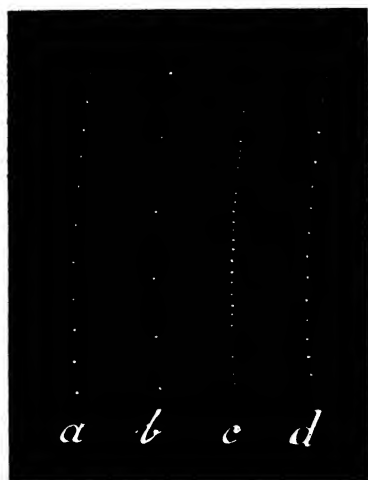


FIG. 67. Effect of application of strong CO_2 on growth of the root of *Vitis quadrangularis*.

- a*. Record of normal rate.
- b*. Preliminary enhancement of the rate after application for one minute.
- c*. Retardation of the rate after 10 minutes.
- d*. Partial recovery on substitution of fresh air.

growth; *b*, the effect of application of strong CO_2 for 1 minute; *c*, the effect of application for 10 minutes; and, finally, *d*, the partial recovery which occurred on substitution of fresh air.

Examination of the series of records clearly indicates that the immediate effect of application of strong CO_2 is an enhancement of the rate; but continued application induces a retardation of the rate; there is, however, a partial recovery towards the normal rate after substitution of fresh air.

The results of this experiment are given below.

TABLE II.—EFFECT OF APPLICATION OF STRONG CO_2 ON GROWTH OF AERIAL ROOT OF *VITIS QUADRANGULARIS*

Normal rate	0.10 μ per second
After application for 1 minute	0.18 μ „ „
„ „ „ 10 minutes	0.02 μ „ „
Sixty minutes after substitution of fresh air	0.07 μ „ „

METHOD OF BALANCE

In order to obtain an independent verification of the results, a far more sensitive method was employed in the following experiment.

Experiment 4. *Determination of the effect of strong CO_2 by the Method of Balance.*—An independent verification of the effect of strong CO_2 on the aerial root of *Vitis quadrangularis* is found from the record obtained on a moving plate, the sensitiveness of which is greatly increased by the Method of Balance. Any enhancement of the rate of growth is indicated by the upsetting of the balance in an upward direction; an induced depression, on the other hand, is shown by the upset of balance in the opposite direction. In the present case the first part of the record N shows the normal rate of growth, this being 0.12 μ per second. After balancing the rate of growth the record B becomes horizontal. On application of strong CO_2 at arrow the balance is upset in an *upward* direction, indicating an enhancement of the rate of growth. But after the continued application of strong CO_2 there is a reversal, indicated by the down-curve, the steepness of the slope of which is seen to increase with the duration of application (fig. 68).

Prolonged application of strong CO_2 thus induces an increasing retardation of the rate of growth.

It will be seen that this Method of Balance gives an independent verification of the effect of strong CO_2 obtained

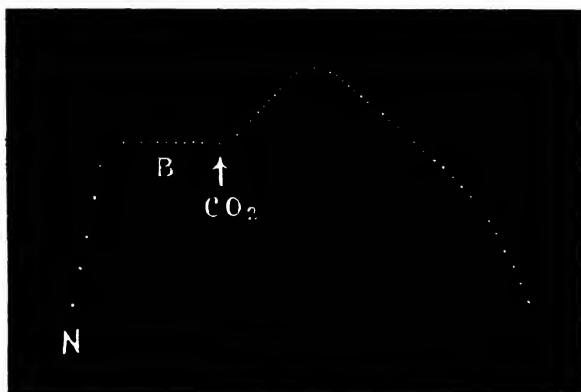


FIG. 68. Effect of strong CO_2 on balanced growth of root of *Vitis quadrangularis*.

N. Record of normal rate.

B. Balanced horizontal record.

Application of CO_2 at arrow induces a preliminary enhancement followed by a marked retardation of the rate of growth.

by the Stationary Plate Method. Both the methods prove that strong CO_2 induces a preliminary enhancement of the rate of growth followed by a marked retardation already described in Experiment 3.

EFFECT OF VAPOUR OF ETHER ON GROWTH OF ROOT OF *VITIS QUADRANGULARIS*

Ether, unless the dose of application is very strong, may be regarded as a mild anæsthetic. The results of numerous experiments show that dilute ether, applied for a moderate length of time, induces an acceleration of the rate of growth; its prolonged application, or the application of a stronger dose, causes, however, a retardation of the rate. The characteristic results will be demonstrated by the two different methods which have already been described.

STATIONARY PLATE METHOD

Experiment 5. *Effect of dilute ether on growth of aerial root.*—The normal rate of growth was 0.18μ per second, represented by *a* in fig. 69. The immediate effect of application of ether is found to be an enhancement of the rate, as demonstrated by the record given in the subsequent



FIG. 69. Effect of ether vapour on growth of the root of *Vitis quadrangularis*.

- a.* Record of normal rate.
- b.* Retarded rate after application for 10 minutes.
- c.* Enhanced rate as an after-effect.

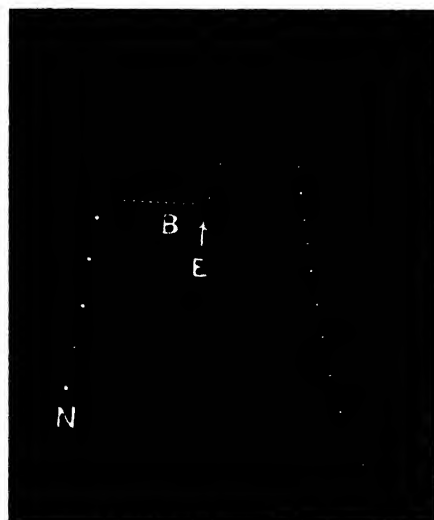
fig. 70. In the present experiment, however, the record *b* was taken after a somewhat prolonged application of the ether vapour, which induced a retardation in the rate of growth. The ether vapour was next removed and fresh air substituted. The remarkable after-effect of previous application of ether in inducing a great enhancement of the rate is seen in the record *c*, the enhanced rate being about one and a half times the normal.

TABLE III.—DIRECT AND AFTER-EFFECT OF DILUTE ETHER VAPOUR ON GROWTH OF ROOT OF *VITIS QUADRANGULARIS*

Normal rate	0·18 μ per second
After application for 10 minutes	0·03 μ „ „
Sixty minutes after substitution of fresh air	0·27 μ „ „

METHOD OF BALANCE

Experiment 6. *Effect of dilute ether on balanced growth of root.*—The normal rate \bar{N} was 0·16 μ per second. After

FIG. 70. Effect of dilute vapour of ether on balanced growth of *Vitis quadrangularis*.

N. Record of normal rate.

B. Balanced horizontal record.

Application of ether, E, at arrow induces a preliminary enhancement of the rate of growth (up-curve) followed by depression (down-curve).

taking the balanced horizontal record B, ether E was applied at arrow. This induced a preliminary enhancement in the rate of growth, as indicated by the upsetting of the balance upwards; continued application of the ether caused,

however, a change from acceleration to retardation, as demonstrated by the reversal of the curve downwards (fig. 70).

EFFECT OF VAPOUR OF CHLOROFORM ON GROWTH OF ROOT OF *VITIS QUADRANGULARIS*

The effect induced under the action of the strong anæsthetic chloroform is not very different from that of the ether, that is to say, the immediate effect of the dilute vapour is an enhancement in the rate of growth followed by a retardation under its prolonged action; this will be presently demonstrated in Experiments 7 and 8. The immediate effect is more easily detected by the method of balance. A strong dose of this anæsthetic is found to be toxic in its action.

STATIONARY PLATE METHOD

Experiment 7. *Effect of dilute chloroform on growth of aerial root.*—The specimen employed for this experiment had

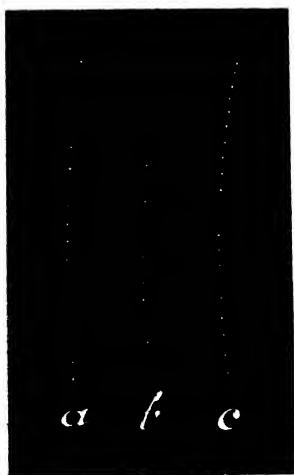


FIG. 71. Effect of dilute chloroform on the growth of the root of *Vitis quadrangularis*.

- a. Record of normal rate.
- b. Retarded rate after application for 10 minutes.
- c. Partial recovery on substitution of fresh air.

the very slow rate of growth of 0.05μ per second. The first series of record, *a*, exhibits the normal rate; the next

series, *b*, taken after a somewhat prolonged application of the chloroform vapour, exhibits a retardation, the rate being reduced to less than half the normal. Substitution of fresh air for the chloroform vapour brought about a partial recovery in the course of half an hour (fig. 71).

TABLE IV.—EFFECT OF DILUTE VAPOUR OF CHLOROFORM ON GROWTH OF ROOT OF *VITIS QUADRANGULARIS*

Normal rate	0.05 μ per second
After application for 10 minutes	0.02 μ " "
Thirty minutes after substitution of fresh air	0.04 μ " "

METHOD OF BALANCE

Experiment 8. *Effect of dilute chloroform on balanced growth.*—The normal rate of growth was in this specimen

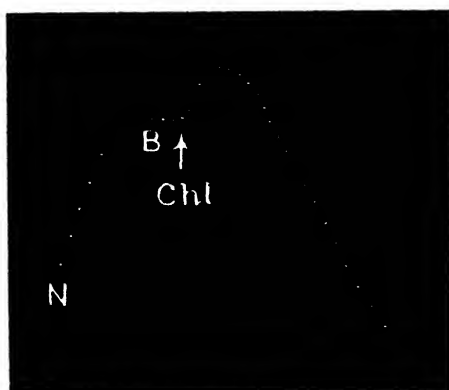


FIG. 72. Effect of dilute chloroform on balanced growth of root of *Vitis quadrangularis*.

N. Record of normal rate.

B. Balanced horizontal record.

Chloroform vapour applied at arrow induces a preliminary enhancement of growth followed by retardation.

0.10 μ per second, represented by N in the first part of the record. After obtaining the balanced horizontal record B, dilute chloroform vapour was applied at arrow. The record

shows a preliminary enhancement of the rate, exhibited by the upsetting of the balance upwards; prolonged application of this narcotic, however, caused a retardation of the rate of growth as demonstrated by the reversal of the curve downwards (fig. 72).

EFFECT OF DILUTE VAPOUR OF AMMONIA ON GROWTH OF THE ROOT OF *VITIS QUADRANGULARIS*

STATIONARY PLATE METHOD

Experiment 9. *Effect of dilute ammonia*.—The normal rate of growth of the root in this specimen was only 0.06μ

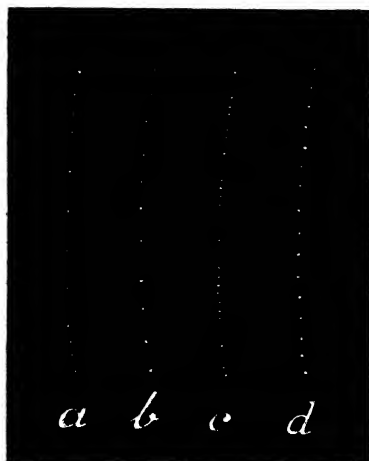


FIG. 73. Effect of dilute NH_3 on growth of the root of *Vitis quadrangularis*.

- a. Record of normal rate.
- b. Preliminary enhancement of the rate after 2 minutes.
- c. Retardation of the rate after 20 minutes.
- d. Recovery on substitution of fresh air.

per second, seen in the first series of the record *a*; after application of vapour of ammonia for two minutes the rate was enhanced to 0.10μ per second, as shown in *b*; after continued application for twenty minutes the rate underwent a great depression, as seen in the series *c*. Fresh air

was next substituted and the series *d* was recorded 60 minutes after the introduction of fresh air; the record shows that not only was there a recovery from the depression, but the rate of growth was even enhanced slightly above the normal to 0.07μ per second (fig. 73).

TABLE V.—EFFECT OF DILUTE AMMONIA VAPOUR ON THE RATE OF GROWTH OF ROOT OF *VITIS QUADRANGULARIS*

Normal rate	0.06μ per second
After application for 2 minutes	0.10μ " "
" " " 20 "	0.02μ " "
Sixty minutes after substitution of fresh air	0.07μ " "

The typical results obtained on normal growth of aerial roots and its induced variations have been described above. The following investigations relate to the responsive variations of the underground roots.

EFFECT OF GASES AND VAPOURS ON GROWTH OF UNDERGROUND ROOT OF *PANDANUS ODORATISSIMUS*

Considerable difficulty was encountered in finding suitable specimens for the purpose of investigations on underground roots. As previously indicated, the necessary condition for success of the experiment depends on finding a main or a lateral root which is stiff enough to produce, during its growth-elongation, an effective pressure on the pan attached to the magnifying system of levers. The particular root for the experiment has to be carefully removed from the soil so as to avoid the shock attending the transfer. In searching for suitable specimens for the experiment it was found that the underground lateral root of *Pandanus odoratissimus* is on the whole the most suitable material for the purpose. The ground is profusely irrigated before the plant is taken out of the soil, so as not to hurt the roots. The lower half of the plant, containing the lateral root, is then detached

from the rest and kept for several hours in a dark moist chamber. After this precaution the growth of the underground root is found to be fully revived. The specimen is then suitably mounted for the record of growth and its induced variations.

Experiment 10. *Uniformity of the rate of growth of underground root under constant external conditions.*—Two series of records were taken to demonstrate the uniformity of the rate of growth under normal conditions, the series *b* being



FIG. 74. Uniformity of rate of growth of underground root of *Pandanus odoratissimus*.

The second record, *b*, was taken half an hour after the first, *a*.

recorded half an hour after the first series *a*. The records show that the rate of growth had practically remained constant during this period (fig. 74). It may be noted here that the rate of growth of this underground root is slower than that of the aerial root of *Vitis quadrangularis*. This necessitates that the successive dots in the record should be taken at intervals of 40 seconds instead of 10 seconds as in previous cases, to prevent their overlapping. The absolute rate of growth in the present case was 0.01μ per second, which is approximately a tenth of the average rate of the aerial root of *Vitis quadrangularis*.

EFFECT OF CO_2 ON GROWTH OF UNDERGROUND ROOT
OF *PANDANUS ODORATISSIMUS*

STATIONARY PLATE METHOD

Experiment II. *Effect of dilute CO_2 on growth of the underground root.*—The first series *a* of the record exhibits the normal rate of growth, which is 0.01μ per second. The



FIG. 75. Effect of dilute CO_2 on growth of underground root of *Pandanus odoratissimus*.

- a. Record of normal rate.
- b. Preliminary enhancement of rate after application for 2 minutes.
- c. Retardation of the rate after 15 minutes.
- d. After-effect on substitution of fresh air.

second series, *b*, taken two minutes after the application of CO_2 , shows enhancement of growth which is nearly twice the normal rate. Continued application for 15 minutes induced a great retardation, seen in the record of series *c*. After the removal of CO_2 the after-effect is not only a complete recovery, but even a slight enhancement of the rate, as indicated in the record of series *d* (fig. 75).

TABLE VI.—EFFECT OF DILUTE CO₂ ON GROWTH OF UNDERGROUND ROOT OF *PANDANUS ODORATISSIMUS*

Normal rate	0.010 μ per second
After application for 2 minutes	0.018 μ " "
" " " 15 "	0.006 μ " "
Sixty minutes after substitution of fresh air	0.011 μ " "

METHOD OF BALANCE

Experiment 12. *Effect of CO₂ on balanced growth.*—The normal rate of growth of the specimen was 0.014 μ per second. The inclined curve in record N represents the

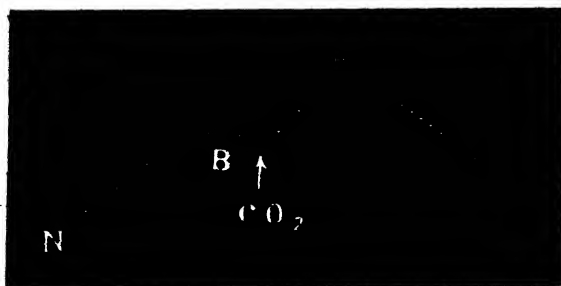


FIG. 76. Effect of dilute CO₂ on balanced growth of root of *Pandanus odoratissimus*.

N. Record of normal rate.

B. Balanced horizontal record.

Application of CO₂ at arrow induces a preliminary enhancement of growth followed by retardation.

normal rate, which being balanced became horizontal at B. The application of CO₂ at arrow produced an upsetting of the balance upwards showing an enhancement of the rate; prolonged application of CO₂, however, brought about a reversal of the curve which indicated an induced retardation of the rate of growth (fig. 76).

EFFECT OF DILUTE ETHER VAPOUR ON GROWTH OF UNDERGROUND ROOT OF *PANDANUS ODORATISSIMUS*

STATIONARY PLATE METHOD

Experiment 13. *Effect of dilute ether on growth of underground root.*—The normal rate of growth of root was 0.01μ per second; this is indicated in series *a* of fig. 77. The

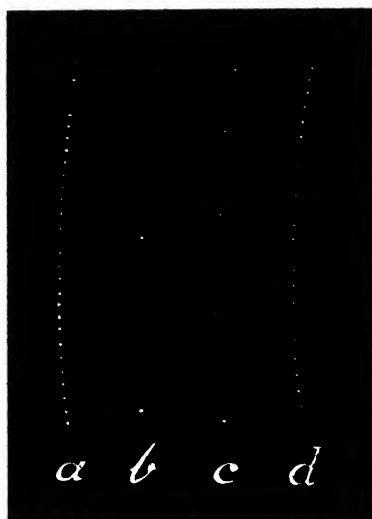


FIG. 77. Effect of dilute ether on growth of root of *Pandanus odoratissimus*.

- a.* Record of normal rate.
- b.* Enhancement of rate after application for 1 minute.
- c.* Persistent enhancement of rate after 5 minutes.
- d.* Effect after application for 15 minutes.

next record, *b*, taken after application of ether vapour for 1 minute, indicated an extraordinarily great enhancement of growth which was fifteen times the normal rate. This enhancement persisted for a considerable length of time; for after application of the anæsthetic for 5 minutes, the record *c* shows that the rate was still ten times the normal. This enhancement underwent a decline under continued application of the ether vapour for 15 minutes; even now the enhancement (record *d*) was about one and a half times the normal rate at the beginning.

TABLE VII.—EFFECT OF DILUTE ETHER ON GROWTH OF UNDERGROUND ROOT OF *PANDANUS ODORATISSIMUS*

Normal rate	0.010 μ per second
After application for 1 minute	0.150 μ „ „
„ „ „ 5 minutes	0.100 μ „ „
„ „ „ 15 „	0.014 μ „ „

METHOD OF BALANCE

Experiment 14. *Effect of dilute ether on balanced growth.*—The results of the previous experiment find a very striking

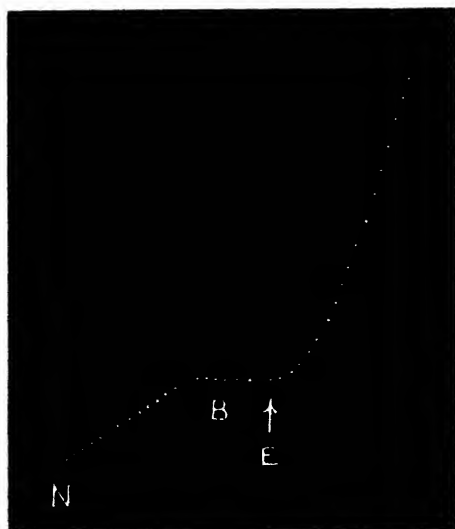


FIG. 78. Effect of dilute ether on balanced growth of root of *Pandanus odoratissimus*.

N. Record of normal rate.

B. Balanced horizontal record.

Application of dilute ether vapour at arrow induces a very marked upsetting of the balance upwards, indicative of an enhancement of the rate of growth which persisted for a long time.

confirmation from the balanced record given in fig. 78. After the normal inclined record N, the balanced horizontal

record B was taken. Application of dilute ether at E is seen to produce a very marked upsetting of the balance upwards, indicative of a great enhancement of the rate of growth, which persisted for a considerable length of time.

EFFECT OF VAPOUR OF AMMONIA ON GROWTH OF UNDERGROUND ROOT OF *PANDANUS ODORATISSIMUS*

STATIONARY PLATE METHOD

Experiment 15. *Effect of vapour of ammonia on growth of the underground root.*—The underground root appears to be greatly affected by the action of ammonia vapour; this is evidenced by the records given in fig. 79 in which the

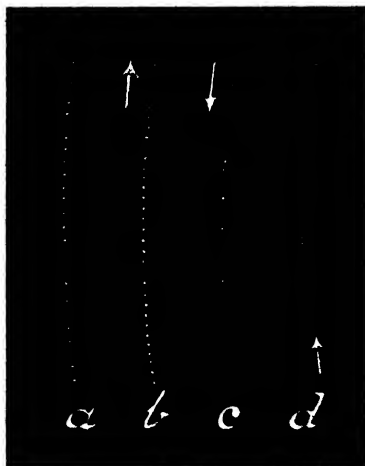


FIG. 79. Effect of dilute NH_3 on growth of underground root of *Pandanus odoratissimus*.

- a. Record of normal rate.
- b. Retarded rate after application for 2 minutes.
- c. Responsive contraction after application for 30 minutes.
- d. Renewal of growth after substitution of fresh air.

Growth-elongation represented in b and d by up-pointing arrow.
Contractile movement in c indicated by down-pointing arrow.

up-moving curve in record a represents the rate of normal growth-elongation, which was found to be 0.011μ per second. After the application of NH_3 for 2 minutes the

second series *b* was recorded, and in this the shortening of the distance between the successive dots indicates an induced retardation of the rate, which now became 0.007μ per second.

Before proceeding further it is necessary to explain the exact significance of the symbols employed in representing the characteristic variation induced in the rate of growth as exhibited in the series of records of fig. 79. The rate of growth-elongation of the root, it should be remembered, is indicated by the rapidity with which the up-curve is effected. Should there be any retardation of the rate of growth, the fact would be indicated by the slowing down of the rate of the ascending curve in which the distance between the successive dots would become shortened. This is exemplified in the record *b*, which exhibits a depression, due to the application of vapour of ammonia for 2 minutes. But prolonged application of NH_3 gave rise to a reaction of an extraordinary character, the *growth-elongation being now transformed into an actual contraction*. This is seen represented in record *c* by the characteristic change of direction of the curve of response. As the growth-elongation in records *b* and *d* is represented by an up-pointing arrow, it is necessary to indicate the reversed contractile movement induced by prolonged application of NH_3 by a down-pointing arrow as in *c*. The rate of this induced contraction is found to be 0.005μ per second. Fresh air was next substituted; this resulted after 60 minutes in the renewal of growth represented by up-pointing arrow in record *d*; the rate of the renewed growth-elongation was, however, still below the normal.

TABLE VIII.—EFFECT OF NH_3 ON GROWTH OF UNDERGROUND ROOT OF *PANDANUS ODORATISSIMUS*

Normal rate	+ 0.011μ per second
After application for 2 minutes	+ 0.007μ " "
" " " 30 "	- 0.005μ " "
Sixty minutes after substitution of fresh air	+ 0.003μ " "

The results are given in detail in the tabular statement on p. 138. In order to differentiate between the growth-elongation and the responsive contraction induced by prolonged action of ammonia, the former is distinguished by a plus and the latter by a minus sign.

EFFECT OF STIMULATION ON THE GROWTH OF ROOTS

The effect of stimulation on the growth of roots was reserved to the end of this Paper. In order to reach a wider generalisation, the experiments were carried out with a large number of roots such as those of *Pandanus* and of *Canna*. The root of *Cicer arietinum* also offered special advantages for investigation.

The results of investigations already carried out at the Institute prove that, in regard to the responsive variations in shoots of plants, the effects of electric and photic stimulations are essentially similar. It was shown further that the effect of light on the growth of shoots is due rather to its action as a stimulus than to its possible photosynthetic action on the chlorophyll.¹ This generalisation will be found to be confirmed by the following experiments on the action of stimulus on the growth of roots in which no chlorophyll is present.

EFFECT OF ELECTRIC STIMULATION ON THE GROWTH OF ROOT

Electric stimulation was applied from an induction coil. A shock of moderate intensity was adjusted and the duration of the shock was maintained constant in all experiments by a tapping key and a metronome interposed in series with the primary coil. The following experiments were carried out with the root of *Pandanus* and of *Cicer*, the electric shock being applied directly on growing region of the root.

Experiment 16. *Effect of electric stimulation on the growth of the root of Pandanus.*—The normal rate of growth

¹ Bose, *Growth and Tropic Movements of Plants*, p. 74; *Transactions Bose Institute*, vol. vii (1931-32), p. 195.

of the root was 0.03μ per second. After obtaining the balanced horizontal record at B, the electric shock was



FIG. 80. Effect of electric stimulation on growth of the underground root of *Pandanus*.

N. Record of normal growth.

B. Balanced horizontal record.

Application of electric shock at S induces a marked upsetting of the curve downwards, indicative of a retardation of the rate of growth.

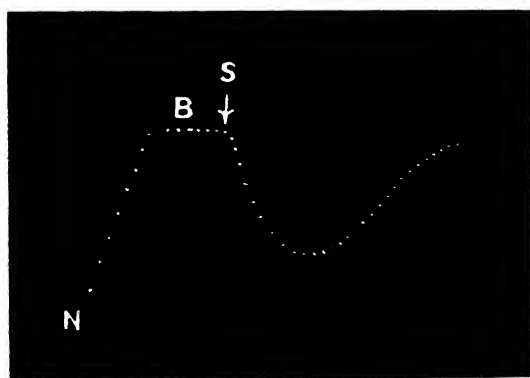


FIG. 81. Effect of electric stimulation on growth of the root of *Cicer*.

N. Record of normal growth.

B. Balanced horizontal record.

Application of electric shock at S.

applied at S. This caused an upsetting of the balance downwards, indicating a retardation of the rate of growth (fig. 80).

Experiment 17. *Effect of electric stimulation on the growth of the root of Cicer.*—The normal rate of growth of the root was 0.052μ per second, which was balanced at B. The electric shock was applied at S, which caused a retardation of the rate of growth, similar to that in *Pandanus* (fig. 81).

EFFECT OF PHOTIC STIMULATION ON THE GROWTH OF ROOT

The following investigations were carried out with roots of *Pandanus odoratissimus*, of *Canna indica* and of *Cicer arietinum*. Proper care was taken that the underground roots of *Pandanus* and *Canna* were not exposed to light when dug out of the soil. The roots were kept in absolute darkness for two or three hours before commencement of the experiment. The *Cicer* seeds were germinated in a dark room, and the roots therefore were protected from any previous action of light.

METHOD OF EXPERIMENT

For observing the effect of photic stimulation on growth the sensitive Method of Balance was employed. Light was obtained from an arc lamp, a thick glass trough with parallel sides and filled with alum solution being interposed in the path of light so as to cut off the heat rays. Two inclined mirrors were placed behind the root so that all sides of the root might be uniformly illuminated.

Experiment 18. *Effect of light on the growth of the root of Pandanus.*—The normal rate of growth of this specimen was 0.042μ per second, which was balanced at B. The application of light at L produced an upsetting of the curve downwards, demonstrating a retardation of the rate of growth. After the withdrawal of light at the arrow within a circle, the rate of growth was gradually restored and became normal as indicated by the horizontal line towards the end of the record (fig. 82).

Experiment 19. *Effect of light on the growth of the root*

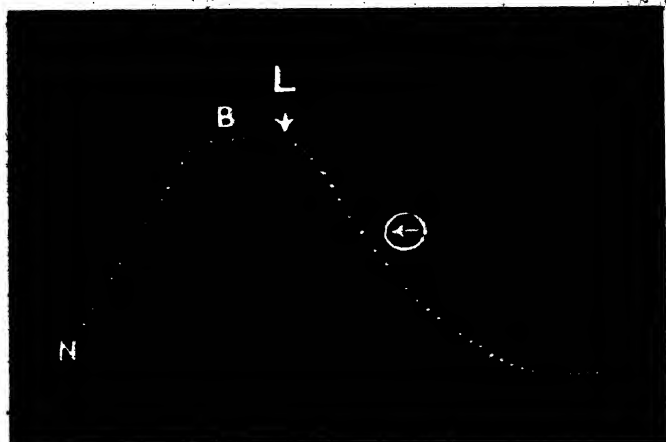


FIG. 82. Effect of light on growth of the underground root of *Pandanus*.

N. Record of normal growth.
B. Balanced horizontal record.

Application of light at L induces an upsetting of the curve downwards, indicating a retardation of the rate of growth. Light withdrawn to arrow within circle is attended by gradual recovery of original rate.

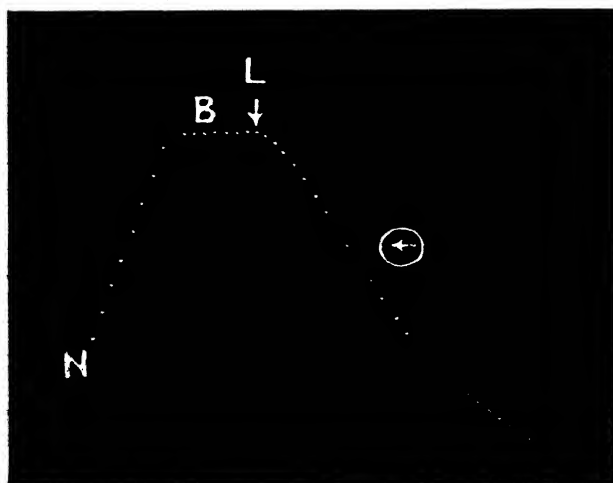


FIG. 83. Effect of light on growth of the underground root of *Canna*.

N. Record of normal growth.
B. Balanced horizontal record.

Application of light at L induces a retardation of the rate of growth.

of *Canna*.—The normal rate of growth was 0.073μ per second. After obtaining balance, the root was subjected to light at L. The upsetting of the curve downwards indicates retardation of the rate of growth (fig. 83).

Experiment 20. *Effect of light on the growth of the root of Cicer*.—The normal rate of growth of this specimen was

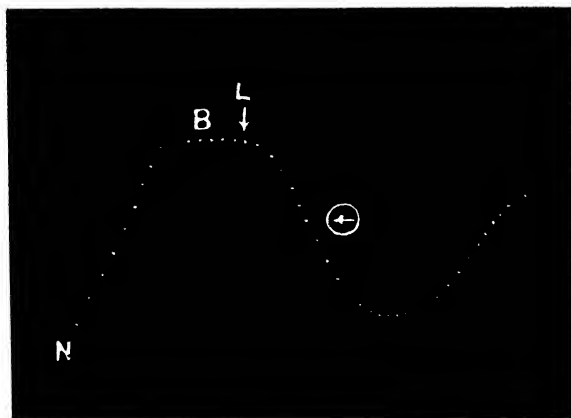


FIG. 84. Effect of light on the growth of the root of *Cicer*.

N. Record of normal growth.

B. Balanced horizontal record.

Light was applied at L and withdrawn at arrow within circle.

0.042μ per second. After obtaining balance light was applied at L, which induced a retardation of the rate of growth as exhibited by the upsetting of the curve in the downward direction. After the withdrawal of light there was not only complete recovery within a short time, but the last portion of the curve turning upwards indicates slight acceleration of the rate of growth above normal, this being an after-effect of the stimulus (fig. 84).

SUMMARY

The object of the present inquiry is the determination of the absolute rate of growth of root and the variation induced in it by different agents.

The automatic record of rate of growth has been obtained from the pressure exerted by the growing root on a suspended aluminium pan. The pan is attached to the short arm of the lever, the long arm of which is connected with the short arm of the second recording lever. By this system of compound levers the growth of root is magnified 4000 times.

The rate of growth of root can thus be determined in the course of a very short time. As the method is automatic the effect of personal errors of observation has been completely eliminated.

The sensitiveness of the new device has been greatly increased by the Method of Balance. When the rate of normal growth is exactly balanced the record is horizontal. An induced enhancement of the rate of growth causes an upsetting of the balance in an upward direction, producing an up-curve in the record. An induced depression, on the other hand, causes a down-curve. The Method of Balance has the unique advantage of determining in a continuous manner the immediate and after-effects of various external agents.

The effect of application of dilute CO_2 on both the aerial and the underground roots is an enhancement of the rate of growth. Prolonged application induces, however, a retardation of the rate.

The effect of very strong CO_2 is a preliminary enhancement of the rate followed by a great depression.

Dilute vapour of ether induces a preliminary enhancement followed by a retardation of growth. On substitution of fresh air the after-effect of previous application of ether is often a very marked enhancement of the rate of growth.

The vapour of ammonia exerts a remarkable action on the underground root. Its immediate effect is a retardation of the rate; but its continued application brings about an active contraction of the root. On the removal of the vapour of ammonia the growth becomes slowly revived.

The effect of stimulation of the root by electric shock of moderate intensity is a retardation of the rate of growth of the organ.

The response to stimulation of the root by light is also

a retardation of the rate of growth; this is essentially similar to that of the electric stimulation.

The results described relate to the determination of the action of certain agencies on the growth of roots by the application of a new method. The same method will undoubtedly open out further lines of investigation on the effect of other agencies in modifying the rate of growth of roots.

VII.—COMPARATIVE STUDY OF THE EFFECT OF DRUGS ON THE RHYTHMIC TISSUES OF ANIMAL AND PLANT

BY

GURU PRASANNA DAS, L.M.S.

THE researches that have already been carried out at the Bose Research Institute prove that the pulsating mechanism in the animal and the plant is, generally speaking, similarly affected by external agencies. In the animal the cardiac tissue represents the automatically pulsating rhythmic tissue. In the plant similar activity is manifested by the pulvinule of the lateral leaflet of *Desmodium gyrans*. In regard to the effect of external agencies on rhythmic activities, it has been shown that variation of temperature, change of internal hydrostatic pressure, the action of narcotics and of poisons affect the rhythmic activity of both animal and plant in an essentially similar manner.

In regard to this the following may be given as concrete examples. In the frog's heart as well as in the pulvinule of *Desmodium gyrans*, fall of temperature is found to induce diminished frequency and increased force of the beat, while rise of temperature produces the opposite effect of increased frequency and diminished force of the beat of the rhythmic pulsating tissues. Again, as in the animal heart so also in the pulsating plant tissue, poisonous solution of *potassium cyanide* is found to produce characteristic arrest of pulsation at the systole by the irreversible change associated with death. In regard to the maintenance of the rhythmic activity in the plant and animal tissues the presence of oxygen is found to be an essential condition.¹

¹ Bose, *Life Movements in Plants*, vol. vi, pp. 78-85.

The present investigation was undertaken to study the comparative effect of several drugs on the rhythmic tissues of the animal and of the plant. These drugs are obtained from certain glands of animals and from some alkaloids derived from plants. It would, no doubt, prove to be of much theoretical interest if certain drugs obtained from such different sources were found to induce similar reactions on rhythmic animal and plant tissues.

The drugs employed for the present investigations are the following :

- (1) *Adrenalin*.—This well-known drug is obtained from suprarenal gland, and is recognised to exert a very marked action on the animal heart.
- (2) *Ephedrin*.—This particular alkaloid is obtained from the plant *Ephedra vulgaris*, found to grow in the Western Himalayas and Afghanistan.
- (3) *Costus speciosus*.—The Bengali name of the plant is *Kur*, the aqueous extract from the root being used for medicinal purposes.

THE METHOD OF RECORD

For investigations on the reactions of the drugs on the animal, the frog's heart was employed for obtaining the record. The apparatus employed was the *Resonant Cardiograph*, specially constructed in the Institute for the present purpose, the successive dots in the record being at intervals of 1/15 of a second. For recording the slower pulsatory activity of the *Desmodium* leaflet and its induced variations, the *Oscillating Recorder* was used, the successive dots in the record being, in this case, at intervals of 2 seconds.

GENERAL REACTION

The effect of a particular drug is characteristically modified by a number of factors. Among these may be mentioned the strength of the dose, the duration of application and the tonic condition of the tissue.

It is thus found that while a feeble dose, applied for a moderate length of time, induces a particular effect, say of stimulation, prolonged application of the same dose induces a depression. A stronger dose, generally speaking, gives rise to a reaction opposite to that of a feeble dose.

In regard to the modifying effect of tonicity on the responsive reaction, the stimulatory effect of a given drug is more marked when the tissue is in a somewhat subtonic condition. For it is obvious that if the pulsating tissue is already in a very favourable tonic condition, the pulsations cannot exhibit any further increase above the optimum.¹

With this preliminary note I proceed to describe the effect of different drugs in greater detail.

EFFECT OF ADRENALIN ON FROG'S HEART

For obtaining reliable results it is advisable to make fresh solution from an ampoule which contains the extract.



FIG. 85.—Effect of dilute *Adrenalin* solution, 1 : 10,000, on heart-beat of Frog.

a. Normal record.

b. Effect of dilute solution in increasing the frequency of beat.
Successive dots at intervals of $\frac{1}{15}$ second.

A solution which is not fresh is liable to decomposition under the action of light and of oxidising agents.

¹ Bose, *Researches on Irritability of Plants* (1913), p. 332.

The extract is supposed to cause an acceleration of the beat of the heart; under certain conditions it causes a slower but a fuller beat. The effect is also, to a certain extent, modified by the dose of application, a small dose inducing an acceleration, while a stronger dose brings about a lowering of the activity.

Experiment 1. *Effect of dilute dose of Adrenalin on frog's heart.*—The particular specimen was in a somewhat subtonic condition. Each complete pulsation is seen to consist of an auricular beat followed by the ventricular. After the application of dilute *Adrenalin*, 1 : 10,000, the frequency of pulsation is seen to undergo a marked increase (fig. 85).

The following table gives a summary of typical results due to the direct application of dilute dose of *Adrenalin* solution, on the force and frequency of heart-beat of Frog.

TABLE I.—EFFECT OF DILUTE DOSE OF ADRENALIN, 1 : 10,000, ON FROG'S HEART

Specimen	Effect of solution	Magnified amplitude in mm.	Frequency per minute
1	Normal	13	40
	After application	13	90
2	Normal	15	70
	After application	15	90
3	Normal	9	90
	After application	10	100

Experiment 2. *Effect of a stronger dose.*—In this case the strength of the dose was increased to 1 : 1000. The effect is shown in fig. 86, in which the application of a stronger dose is seen to decrease the frequency, while producing a marked increase of the force of the beat.



FIG. 86. Effect of a stronger dose of *Adrenalin* solution, 1 : 1000, on heart-beat of Frog.

a. Normal record.

b. Effect of strong solution in increasing the force and diminishing the frequency of beat.

The following table exhibits some typical results, on the effect of application of a stronger dose of *Adrenalin* solution, on the force and frequency of heart-beat of Frog.

TABLE II.—EFFECT OF STRONGER DOSE OF *ADRENALIN*, 1 : 1000, ON FROG'S HEART

Specimen	Effect of solution	Magnified amplitude in mm.	Frequency per minute
1	Normal . After application .	13 22	75 45
2	Normal . After application .	11 14	100 45
3	Normal . After application .	12 18	75 56

Investigation on the effect of *Adrenalin* on *Desmodium* pulsation was next undertaken in order to find out whether there is any resemblance between the effect of *Adrenalin* on the rhythmic tissue of the plant and on the cardiac tissue of the animal.

EFFECT OF ADRENALIN ON THE PULSATION OF THE *DESMODIUM* LEAFLET

Two different methods were employed for the application of the drug. In one case it was *directly* applied on the

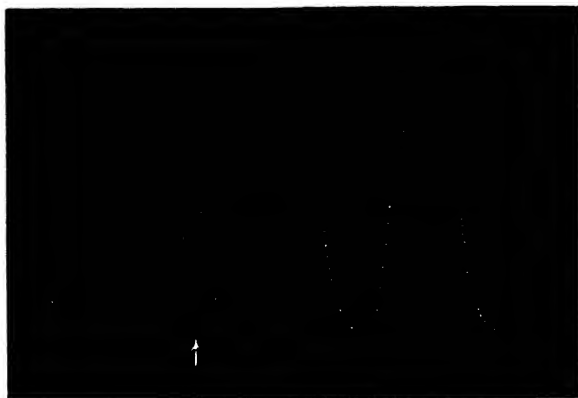


FIG. 87. Effect of direct application of *Adrenalin* solution, 1 : 1000, on the pulsation of *Desmodium gyrans*.

The first two pulsations are normal. *Adrenalin* solution applied at arrow induced increase of amplitude and diminution of frequency of beat.

Successive dots in records of *Desmodium* at intervals of 2 seconds.

pulvinule of the leaflet. In the second case the drug was applied not on the pulsating organ itself but *indirectly* at a distance from the motor organ. I will first describe the effect of direct application of the drug, the strength of the dose being 1 : 1000.

Experiment 3. *Effect of direct application of Adrenalin on the pulvinule of leaflet.*—The effect is seen recorded in fig. 87, in which the first two pulsations are normal, and the

TABLE III.—EFFECT OF *DIRECT APPLICATION OF ADRENALIN*,
1 : 1000, ON THE FORCE AND FREQUENCY OF PULSE-BEAT
OF *DESMODIUM GYRANS*

Specimen	Effect of solution	Magnified amplitude in mm.	Frequency per hour
1	Normal . . . After application .	19 41	18 9
2	Normal . . . After application .	23 39	34 14
3	Normal . . . After application .	13 20	34 21
4	Normal . . . After application .	9 19	26 13
5	Normal . . . After application .	28 35	23 16
6	Normal . . . After application .	25 32	30 20
7	Normal . . . After application .	32 41	30 22
8	Normal . . . After application .	16 30	30 20

last two exhibit the effect of direct application of *Adrenalin*. It will be noticed that the result of application of a moderately strong dose on the pulsation of the leaflet is essentially

similar to that on the heart of the frog ; that is to say, a lowering of frequency but an increase of the force of the beat. The effect of the drug became quite evident about 2 minutes after the application.

Table III gives a summary of typical results on the effect of direct application of *Adrenalin*, 1 : 1000, on the force and frequency of pulsation of the rhythmic tissue of the pulvinule of *Desmodium*.

After describing the effect of *direct* application of the drug, I proceed to explain the method of *indirect* application and the results induced.

EFFECT OF INDIRECT APPLICATION

The drug is said to act indirectly when the solution is applied at the distant cut end of the petiole bearing the pulsating leaflet. In this case it is by the transport of the solution, through the intervening distance by the ascent of sap, that the drug reaches the pulsating pulvinule.

A diagrammatic representation of the method of indirect application of the drug, also of the Oscillating Recorder, is shown in fig. 88. The petiole of the leaflet to be experimented upon is held in position by a sliding holder. The cut end of the petiole dips to begin with in a cup of water, *c*. The cup containing water is quickly replaced by a similar cup containing the solution of requisite strength of the drug whose effect is to be studied. The leaflet is attached to a fine cocoon thread by a drop of shellac varnish, the thread itself being tied to the short arm of a magnifying writing lever W, dots being inscribed on an oscillating smoked glass plate G, the successive dots inscribed being at intervals of 2 seconds.

Experiment 4. *Effect of indirect application of Adrenalin, 1 : 1000, on the cut end of the petiole of Desmodium.*—The effect of indirect application at the cut end of the petiole is seen in the record (fig. 89). When the solution was thus applied, not on the pulsating organ itself but at the distant cut end of the petiole, the characteristic effect occurred somewhat later. Under direct application the result was

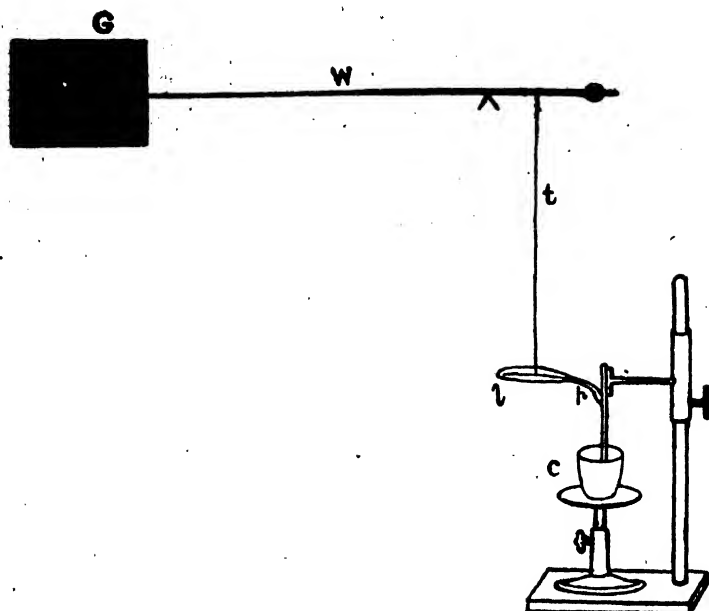


FIG. 88. Diagrammatic representation of the *Oscillating Recorder* and the method of indirect application of the drug.

The pulsating leaflet *l* is attached by means of a cocoon thread *t* to the short arm of the writing lever *w*; *p*, the pulsating pulvinule; *c*, cup for supplying water or the desired solution to the cut end of the petiole; *G*, smoked glass plate.



FIG. 89. Effect of indirect application of *Adrenalin* solution, 1 : 1000, at the cut end of the petiole of *Desmodium*.

The first pulsation is normal; on application of *Adrenalin* at arrow, there was a staircase increase of amplitude of pulsation.

manifested within 2 minutes, but in the present case it took place after 5 minutes. The delay is due to the fact that the solution had to be transported by the ascent of sap through the intervening distance to the pulsating pulvinule for inducing the characteristic result.

This effect of indirect application is, however, an enhancement of the pulsatory activity exhibited by an increased amplitude of pulsation. Direct application of a moderately strong solution of 1 : 1000 is, however, found to induce a depression, as demonstrated in Experiment 3.

The probable explanation is that the solution which reaches the pulvinule becomes attenuated by the transport through the intervening distance, the result being tantamount to the action of a more dilute dose, which has been shown to induce a stimulatory action. The following table gives a summary of the results of other experiments.

TABLE IV.—EFFECT OF INDIRECT APPLICATION OF ADRENALIN SOLUTION, 1 : 1000, ON THE PULSATION OF *DESMODIUM*

Specimen	Effect of solution	Magnified amplitude in mm.	Frequency per hour
1	Normal	4	20
	After application	44	26
2	Normal	10	24
	After application	20	26
3	Normal	11	15
	After application	25	16

It has been stated previously that the stimulatory effect of the drug becomes very marked when the organ, owing to subtonicity, is in a more or less quiescent condition. An example of this is given below.

Experiment 5. *Effect of Adrenalin on a quiescent leaflet*

of *Desmodium*.—The record (fig. 90) shows that the leaflet to begin with was practically in a quiescent condition. But

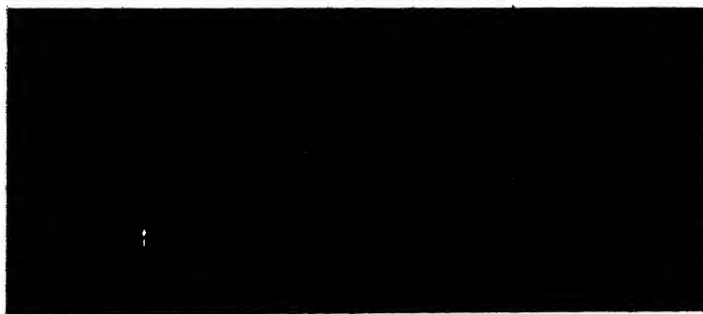


FIG. 90. Effect of indirect application of *Adrenalin* on a quiescent leaflet of *Desmodium*.

The wavy line at the beginning indicates that the pulsatory activity of the leaflet was practically at a standstill. Application of *Adrenalin* at arrow brings about a marked enhancement of amplitude of pulsation.

indirect application of *Adrenalin* brought about a renewal of activity, which became vigorous in the course of a short time.

EPHEDRIN

The alkaloid *Ephedrin* was first isolated by Nagai from the plant *Ephedra vulgaris*. This drug has the advantage of being more stable and does not decompose so readily as *Adrenalin*. In its pharmacological action it resembles *Adrenalin*, and its effect persists for a longer time. The effect of the drug depends on the strength of the solution, a stronger dose inducing an effect opposite to that of a feeble dose.

EFFECT OF EPHEDRIN ON FROG'S HEART

The solution of requisite strength was prepared immediately before the experiment and applied on the heart.

Experiment 6. *Effect of Ephedrin on the heart of the Frog*.—With a very dilute solution the effect was not very marked. A one per cent. solution, however, was found to

induce a very marked increase of force and diminution of frequency, as will be seen from the record (fig. 91). The effect, moreover, persisted for a very long time.



FIG. 91. Effect of *Ephedrin* solution, 1 : 100, on Frog's heart.

a. Normal record.

b. After application of *Ephedrin*.

Note resulting increase of force and diminution of frequency.

The following table contains certain typical results on the effect of direct application of *Ephedrin* solution on Frog's heart.

TABLE V.—EFFECT OF APPLICATION OF *EPHEDRIN*, 1 : 100, ON FROG'S HEART

Specimen	Effect of solution	Magnified amplitude in mm.	Frequency per minute
1	Normal	10	75
	After application	20	45
2	Normal	14	70
	After application	24	41

The effect of the same drug in inducing changes in the pulsatory activity of the leaflet of *Desmodium gyrans* was next studied.

EFFECT OF EPHEDRIN ON THE PULSATION OF *DESMODIUM* LEAFLET

The solution was applied directly on the pulvinule and the changes induced were recorded.

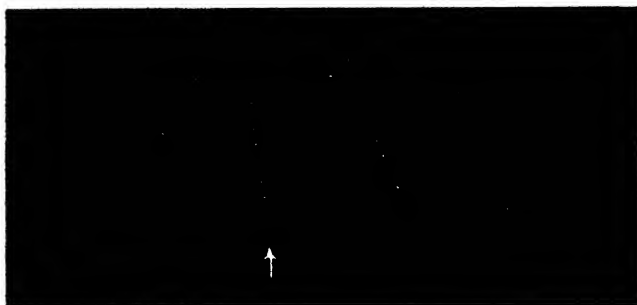


FIG. 92. Effect of direct application of *Ephedrin*, 1 : 500, on *Desmodium* pulsation.

The first two pulsations are normal; subsequent pulses, after application of *Ephedrin*, show increased force and diminished frequency of pulsation.

TABLE VI.—EFFECT OF DIRECT APPLICATION OF *EPHEDRIN*, 1 : 500, ON *DESMODIUM* PULSATION.

Specimen	Effect of solution	Magnified amplitude in mm.	Frequency per hour
1	Normal	20	16
	After application	25	8
2	Normal	12	30
	After application	18	24

Experiment 7. *Effect of direct application of Ephedrin on the pulvinule of Desmodium.*—The first two pulsations are normal (fig. 92). On the application of *Ephedrin*

solution, 1 : 500, at arrow, an increase of force of the beat and diminished frequency occurred in the course of 2 minutes.

Table VI gives typical results on the effect of direct application of *Ephedrin* solution on the force and frequency of beat of the leaflet of *Desmodium gyrans*.

COSTUS SPECIOSUS

This plant belongs to the natural order *Zingiberaceae*. Its Bengali name is *Kur*. It is a climbing plant found abundantly in Bengal and in Cashmere.

In the following experiments, aqueous extract from the root was used.



FIG. 93. Effect of application of aqueous extract of *Costus*, 1 : 10, on Frog's heart.

- a. Normal record.
- b. After application of *Costus*.

Note increased force and diminished frequency of heart-beat.

Experiment 8. *Effect on Frog's heart*.—On application of a ten per cent. aqueous solution there was induced an increase of force of the beat and a diminution of frequency (fig. 93). The effect occurred in the course of 1 minute after application.

The following table gives typical results of the application of the root-extract of *Costus speciosus* on Frog's heart.

TABLE VII.—EFFECT OF APPLICATION OF THE AQUEOUS EXTRACT, 1 : 10, OF *COSTUS* ON THE FORCE AND FREQUENCY OF FROG'S HEARTBEAT

Specimen	Effect of solution	Magnified amplitude in mm.	Frequency per minute
1	Normal	11	70
	After application	17	43
2	Normal	10	70
	After application	19	36
3	Normal	6	90
	After application	12	53

Investigations were next undertaken on the effect of this drug on the pulsatory activity of *Desmodium* leaflet.

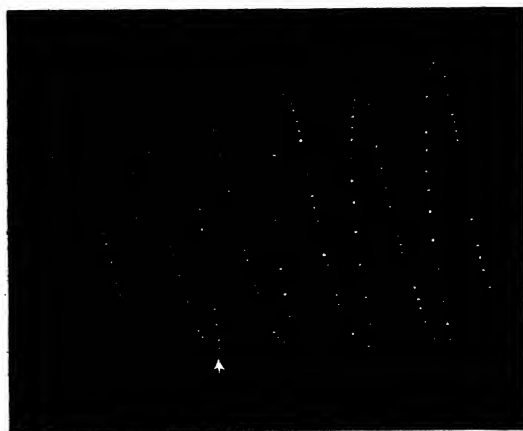


FIG. 94. Record showing effect of direct application of *Costus*, 1 : 10, on the pulsation of *Desmodium* leaflet.

The first two pulsations are normal. Application of *Costus* at arrow induced increased force and diminished frequency.

TABLE VIII.—EFFECT OF *DIRECT* APPLICATION OF EXTRACT OF *COSTUS*, 1:10, ON THE PULSATION OF *DESMODIUM* LEAFLET

Specimen	Effect of solution	Magnified amplitude in mm.	Frequency per hour
1	Normal	27	15
	After application	39	12
2	Normal	13	30
	After application	25	15
3	Normal	18	17
	After application	30	15
4	Normal	13	30
	After application	27	22
5	Normal	11	30
	After application	26	20
6	Normal	11	30
	After application	21	17

EFFECT OF EXTRACT OF *COSTUS SPECIOSUS* ON
DESMODIUM PULSATION

The aqueous extract was applied directly on the pulvinule and the effect induced was recorded.

Experiment 9. *Effect of direct application of extract of Costus, 1:10, on the pulvinule of Desmodium leaflet.*—Direct application of the extract of the same strength as was used in the preceding experiment on frog's heart was found to induce a very similar effect, being an increase of the force of the beat and a diminution of the frequency (fig. 94).

The result of the extract became evident in the course of 2 minutes after the application.

Table VIII gives an account of typical results on the effect of direct application of extract of *Costus*, 1 : 10, on pulsation of the leaflet of *Desmodium*.

SUMMARY

Experiments are described demonstrating the characteristic effects of certain drugs on the rhythmic tissues of the animal and the plant.

It is shown that these drugs, derived from different sources, induce effects which are essentially similar on the pulsatory activity of the heart of the frog and on that of the leaflet of *Desmodium gyrans*. The direct effect of moderately strong doses of *Adrenalin*, *Ephedrin* and of *Costus* is an increase of force of the beat and a diminution of frequency of pulsation.

In the case of the leaflet of *Desmodium gyrans* its rhythmic activity undergoes a definite modification when the drug is applied indirectly at the cut end of the petiole bearing the leaflet. In this case the drug is transported, by the ascent of sap, through the intervening distance to the pulsating pulvinule, causing a definite modification of the activity, characteristic of the application of a dilute dose.

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VIII.—STUDY OF THE ACTION OF CERTAIN DRUGS ON DIFFERENT SEGMENTS OF THE ALIMENTARY TRACT

BY

NOGENDRA NATH DAS, M.B., M.Sc.

A NUMBER of investigations carried out at the Institute gave demonstrations of the characteristic reactions of different Indian drugs on the frog's stomach.¹ The present inquiry was undertaken to determine whether a particular drug induced the same kind of reaction on different sections of the alimentary canal, or whether these segments responded differently. The alimentary canal may conveniently be divided into the following three segments :

- (1) The Oesophagus.
- (2) The Stomach.
- (3) The upper portion of the Intestine.

PREPARATION OF THE SPECIMEN AND METHOD OF RECORD

The alimentary tract of the pithed frog was separated from the adjoining viscera, and different segments dissected out, the isolated pieces being kept in normal saline in separate vessels for subsequent experiments.

The peristaltic activity was recorded by the *Automatic Peristaltograph*, which was specially devised for this purpose. The apparatus consists of a fixed V-shaped rod and a movable vertical lever ; the preparation of one of the sections

¹ N. N. Das, 'Investigations on Effect of Certain Indian Drugs on Frog's Stomach,' *Transactions Bose Research Institute*, Vol. vii.

of the alimentary tract was placed between the V-shaped rod and the movable lever, and kept in position by means of two pins. The passage of the peristaltic wave moves the primary lever either inwards or outwards. The smallest movement is further magnified by a small wheel of about 1 cm. in diameter, which is made to rotate in one direction or the other by the peristaltic expansion or contraction of the stomach. A long writing lever is attached to the rotating wheel (fig. 95); this marks successive dots at short

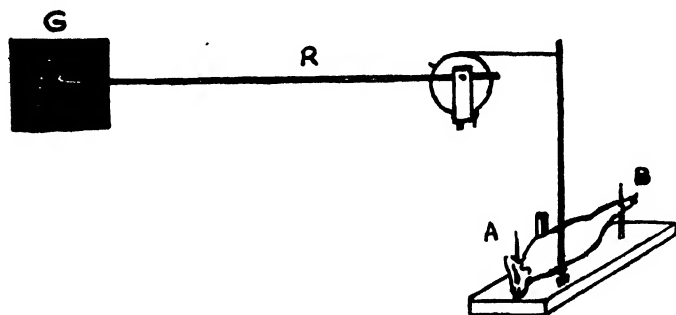


FIG. 95. The diagrammatic representation of *Peristaltograph*

The tissues, viz. oesophagus, stomach or intestine, are fixed by two pins, A, upper or oesophageal end, B, lower or pyloric end. The vertical rod is mounted on a short length of spring, so that it moves inwards or outwards by contraction or expansion of the tissues; the upper end of rod is attached to a small wheel carrying the long recording lever, R; smoked glass plate, C.

intervals of 1 or 2 seconds, by means of an electro-magnetic device. The compound magnification produced by the primary and the recording lever is either 100 or 200 times. The first is quite sufficient for general purposes, while the second is employed for record of relatively feeble pulsations.¹

The investigations described in this paper were carried out with extracts obtained from the following plants:

- I. *Costus speciosus*.
- II. *Adenanthera pavonina*.
- III. *Alstonia scholaris*.

¹ For detailed description of the *Peristaltograph* cf. *Transactions of the Bose Research Institute*, Vol. vii, p. 222.

- IV. *Illicium verum*.
- V. *Lepidium sativum*.
- VI. *Rhus succedanea*.
- VII. *Rubia cordifolia*.

The effects of an individual drug were studied on different sections of the alimentary tract, and their characteristic reactions recorded.

I. *COSTUS SPECIOSUS*

General description and method of preparation of the extract.—The Bengali synonym of this plant is *Kur*; it belongs to the N.O. *Zingiberaceae*. The roots and the tubers are generally used for medicinal purposes. It has slightly bitter taste and strong aromatic odour. The roots, which are dark brown in colour, were used for obtaining the extract. One gram of the root is thoroughly macerated in a mortar

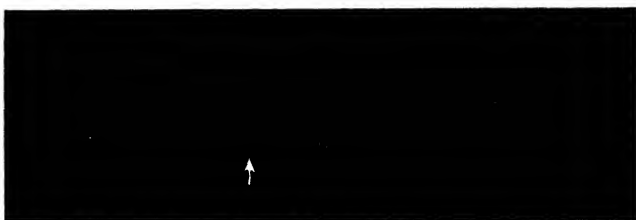


FIG. 96. Effect of aqueous extract of *Costus* on oesophagus. The extract applied at arrow induces practically no change.

with 10 c.c. of normal saline. After soaking for one hour the infusion was filtered, and the filtrate used for the experiment.

Experiment 1. Effect of extract from Costus on the Oesophagus.—The isolated preparation of the oesophagus of the frog was suitably mounted in the *Peristaltograph*. After taking the record of normal activity the aqueous extract was applied on the oesophagus at arrow; this was found to induce practically no change (fig. 96).

Experiment 2. Effect of extract from Costus on the Stomach.—The normal peristalsis of the stomach was found

to be fairly active, and on the application of the drug there was induced an immediate enhancement. This was, how-



FIG. 97. Effect of aqueous extract of *Costus* on stomach. Normal peristalsis was fairly active. Application of the drug at arrow induced an enhancement, followed by great depression, as indicated by enfeebled pulses and longer periods of intermediate pause.

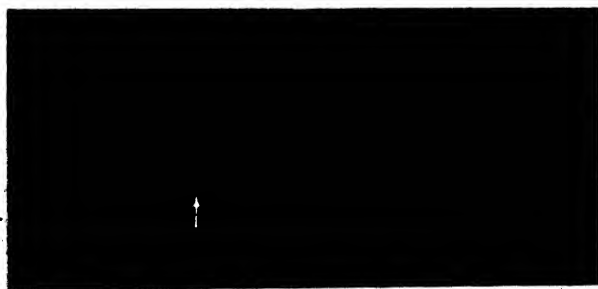


FIG. 98. Effect of aqueous extract of *Costus* on intestine. Normal feeble activity was followed, after application of the drug, by great enhancement, which was, however, short-lived.

ever, followed by a great depression, the pulsations becoming enfeebled, with longer periods of intermediate pause (fig. 97).

Experiment 3. *Effect of extract from Costus on the Intestine.*—The general activity of the intestine was very feeble; but the application of the drug induced a great enhancement of its activity. This enhancement, however, was short-lived, being followed by a great depression (fig. 98).

The experimental results given above prove that the application of the same drug on different sections of the alimentary tract induces not the same but dissimilar reactions.

II. *ADENANTHERA PAVONINA*

The Bengali synonym of this plant is *Raktakambal*. The seeds, leaves and roots are generally used for medicinal purposes. The seed is purple-red in colour, bi-convex in shape and weighs about 3 grains. When soaked in water for several hours, the hard seed coat bursts with a jerk,

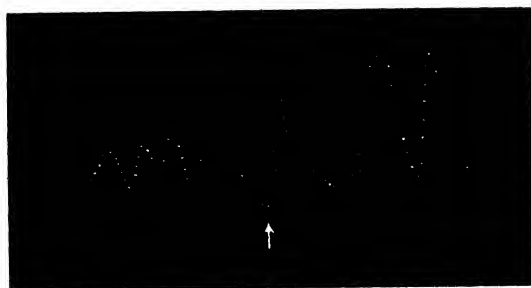


FIG. 99. Effect of aqueous extract of *Adenanthera* on oesophagus. Normal pulses were feeble. After application of the drug the pulses became larger and more forceful.

accompanied by a crackling sound. In preparing the extract the dried seeds are powdered, one gram of the powder being mixed with 10 c.c. of normal saline and left for half an hour. The filtrate is yellowish white and smells somewhat like castor oil.

Experiment 4. *Effect of extract from Adenanthera pavonina on the Oesophagus.*—After mounting the oesophagus on the *Peristaltograph*, the normal record was taken. The drug was then applied drop by drop, and the change induced is shown in the record (fig. 99). The normal oesophageal

pulses, as indicated by their amplitudes, were very feeble ; on the application of the drug, however, at arrow the pulses became enhanced in amplitude and exhibited considerable force. This particular reaction persisted for more than an hour and a half.

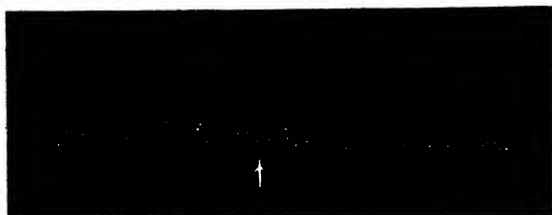


FIG. 100. Effect of aqueous extract of *Adenanthera* on stomach. On application of the drug there was a transient enhancement of activity followed by depression.

Experiment 5. *Effect of extract from Adenanthera on the Stomach.*—On application of the drug at arrow there was induced a transient enhancement of activity followed by a great depression (fig. 100).

Experiment 6. *Effect of extract from Adenanthera on the Intestine.*—The same drug was applied on the specimen of

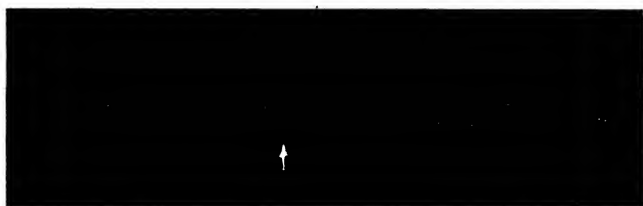


FIG. 101. Effect of aqueous extract of *Adenanthera* on intestine. After application of the extract at arrow the feeble normal pulsations became slightly enhanced.

isolated intestine and the induced changes recorded. The normal pulses were very feeble, but after application of the drug at arrow there was a slight enhancement of activity (fig. 101).

In the case of the drug *Adenanthera*, the effects induced at different parts of the alimentary tract are not exactly the same, but characteristically different as in *Costus*.

III. *ALSTONIA SCHOLARIS*

This plant belongs to the natural order *Apocynaceae*. The Bengali name is *Chhatim*. The drug was obtained from the bark, one gram of the bark being macerated in 10 c.c. of normal saline.

Experiment 7. *Effect of extract from Alstonia on the*

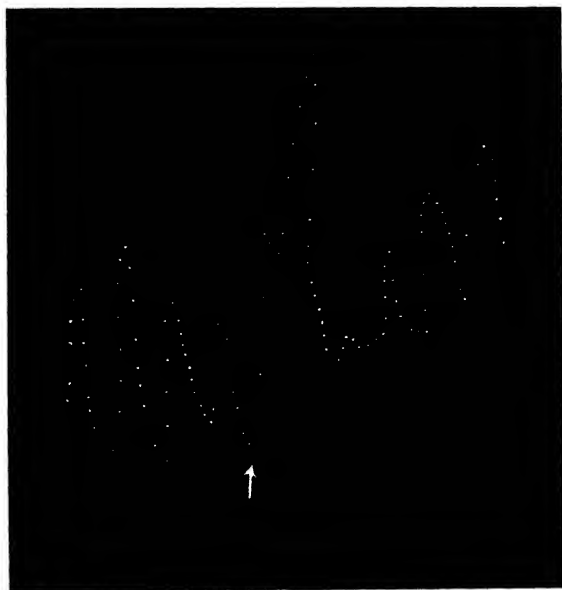


FIG. 102. Effect of aqueous extract of *Alstonia* on oesophagus.

On application of the extract there was a marked stimulatory effect. The oesophagus became somewhat contracted, but the pulsatory activity was nevertheless maintained.

Oesophagus.—The pulsations were fairly active in this particular specimen. After application of the drug a very marked stimulatory effect was induced; there was a tendency for the oesophagus to become contracted, as seen by the displacement of the curve upwards. The pulsatory activity was nevertheless maintained even in this contracted condition (fig. 102).

Experiment 8. *Effect of extract from Alstonia on Stomach.*

—On application of the extract there was a marked stimulation with contraction; the organ, however, exhibited a gradual recovery from contraction, during which period the induced enhancement of activity persisted (fig. 103).



FIG. 103. Effect of aqueous extract of *Alstonia* on stomach. There was stimulation and temporary contraction of the organ.

Experiment 9. *Effect of extract from Alstonia on Intestine.*
—The normal pulsations were feeble. After application of the drug the pulsations became slightly enhanced (fig. 104).



FIG. 104. Effect of aqueous extract of *Alstonia* on intestine. Normal pulses were feeble, but after the application of the drug there was induced a slight enhancement of the activity.

IV. *ILLCIUM VERUM*

This plant is commonly known as *Badian* in Bengali. Its fruit contains a volatile oil, sugar, a bitter principle and tannin.

The fruits were used for obtaining the extract. One gram of the fruit was thoroughly macerated in a mortar with 10 c.c. of normal saline. After soaking for one hour the



FIG. 105. Effect of aqueous extract of *Illicium verum* on oesophagus.

Normal feeble pulses exhibit slight enhancement of activity after application of the drug.



FIG. 106. Effect of aqueous extract of *Illicium verum* on stomach.

After application of the drug there was a transient stimulation followed by return to the normal.

infusion was filtered, and the filtrate was used for the experiment.

Experiment 10. *Effect of extract from Illicium on Oesophagus.*—After application of the drug the normal feeble pulses became moderately enhanced in activity (fig. 105).

Experiment 11. *Effect of extract from Illicium on the Stomach.*—The stomach was mounted in the usual way and normal record obtained. After application of the drug there was induced an enhancement of activity. This enhancement was, however, short-lived, the pulses becoming normal after a short time (fig. 106).

Experiment 12. *Effect of extract from Illicium on the Intestine.*—The intestine in the present case was very inactive. But after application of the drug at arrow, there

FIG. 107. Effect of aqueous extract of *Illicium verum* on intestine.

The quiescent intestine became activated after application of the drug, the enhanced activity persisting for a long time.

was induced a marked enhancement of activity which persisted for a very long time, as exhibited in the two succeeding series of records (fig. 107).

V. *LEPIDIUM SATIVUM*

This plant belongs to the N.O. *Cruciferae*. In Hindi it is known as *Halim*. Its seeds, containing volatile aromatic oil, are used for medicinal purposes. One gram of the powdered seeds was soaked in 10 c.c. of normal saline and the infusion filtered after half an hour. The colour of the filtrate was yellow white.

Experiment 13. *Effect of extract from Lepidium on Oesophagus.*—On application of the extract the oesophagus became contracted; the recovery from this contracted condition was effected by a series of small pulses (fig. 108).

Experiment 14. *Effect of extract from Lepidium on Stomach.*—The application of the drug at arrow induced a

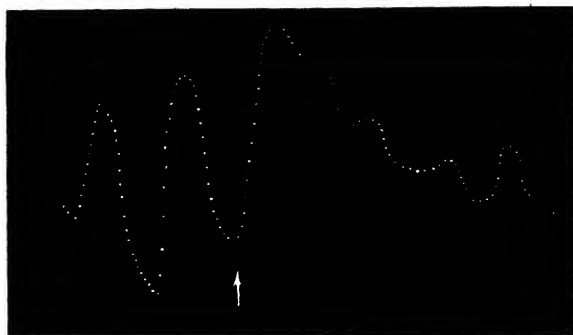


FIG. 108. Effect of aqueous extract of *Lepidium* on oesophagus. After application of the extract there was induced a contraction of the organ from which there was a recovery by a series of small pulses.

very marked enhancement of activity. This was, however, of a transient nature, as seen in the record (fig. 109).

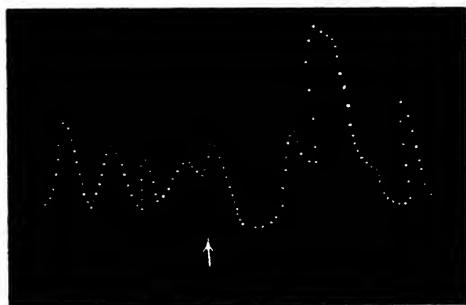


FIG. 109. Effect of aqueous extract of *Lepidium* on stomach. First portion of the record shows normal pulsation. The drug was applied at arrow; this induced a temporary enhancement of activity.

Experiment 15. *Effect of extract from Lepidium on Intestine.*—The activity of the intestine after the application

of the extract became more regular and enhanced; this persisted for a considerable length of time, as indicated by the two series of records (fig. 110).

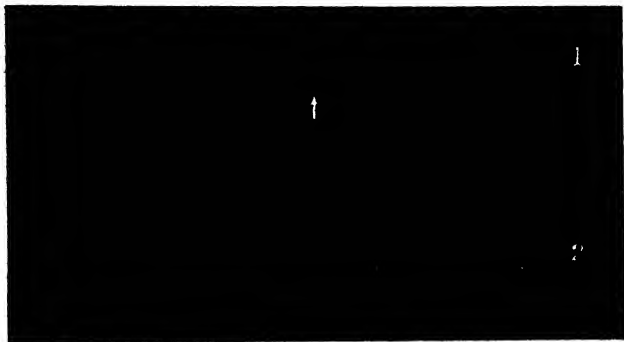


FIG. 110. Effect of aqueous extract of *Lepidium* on intestine. After application of the drug the pulsations became more regular and forceful.

VI. *RHUS SUCCEDANEA*

The plant belongs to the N.O. *Anacardiaceae*, its Bengali name being *Kakrasringi*, from its supposed resemblance to a small horn. It is a gall with hollow hard thin wall tapering at either extremity. It contains a high percentage of tannin as well as an aromatic oil.

The extract is prepared by thoroughly macerating one gram of the substance with 10 c.c. of normal saline. The filtrate used for the experiment is pale yellow in colour.

Experiment 16. *Effect of extract from Rhus succedanea on the Oesophagus*.—After mounting the oesophagus normal record was taken. The organ was in a quiescent condition; the application of the extract induced a short-lived stimulatory reaction (fig. 111).

Experiment 17. *Effect of extract from Rhus succedanea on the Stomach*.—The normal activity was very vigorous; after the application of the extract there was a transient enhancement followed by a depression and arrest of pulsation (fig. 112).



FIG. 111. Effect of aqueous extract of *Rhus succedanea* on oesophagus.

Normally oesophagus was quiescent; after application of the extract there was a transient enhancement.

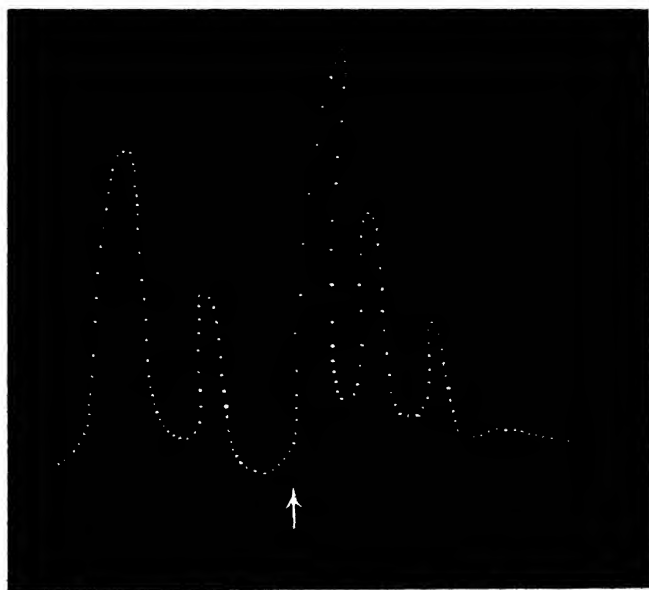


FIG. 112. Effect of aqueous extract of *Rhus succedanea* on stomach.

Normal pulses were vigorous; on application of the extract a transient enhancement was followed by depression.

Experiment 18. *Effect of extract from Rhus succedanea on the Intestine.*—The normal feeble pulsations became more forceful after the application of the extract (fig. 113).



FIG. 113. Effect of aqueous extract of *Rhus succedanea* on intestine.

Normal feeble pulses became more forceful after application of the extract.

VII. *RUBIA CORDIFOLIA*

The Bengali name of the plant is *Manjista*. It contains a colouring matter known as purpurin. When the decoction is used as a medicine it tinges the blood and urine. The



FIG. 114. Effect of aqueous extract of *Rubia cordifolia* on oesophagus.

Feeble pulsations became considerably enhanced after application of the extract. The increased activity persisted for a long time.

extract is obtained from dried stems, one gram of the stem being soaked in 10 c.c. of normal saline.

Experiment 19. *Effect of extract from Rubia cordifolia on the Oesophagus.*—The activity of the pulsations of the

oesophagus was feeble. But after the application of the drug the pulsations became gradually enhanced till a maximum amplitude was reached, as shown in the second series of record. This enhancement persisted for a considerable length of time (fig. 114).

Experiment 20. *Effect of extract from Rubia cordifolia*

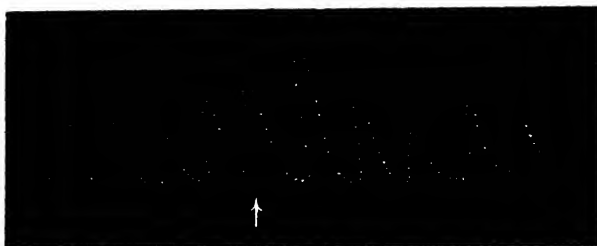


FIG. 115. Effect of aqueous extract of *Rubia cordifolia* on stomach.

The application of the drug at arrow induced a transient enhancement of activity.

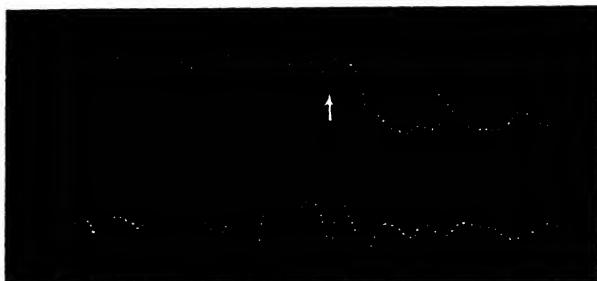


FIG. 116. Effect of aqueous extract of *Rubia cordifolia* on intestine.

Normal pulses were feeble, and on application of the extract there was a relaxation, shown by the displacement of the curve in a downwards direction.

on the Stomach.—The normal activity of the stomach was moderate; the application of the drug induced a transient enhancement of activity, as seen in the record (fig. 115).

Experiment 21. *Effect of extract from Rubia cordifolia on the Intestine.*—The drug acted on the intestine almost in the same way as on the oesophagus. The feeble pulses

became enhanced after application of the drug. The action was more lasting than that on the stomach.

The noticeable effect of the extract on the intestine is the induced relaxation shown by the displacement of the curve in a downward direction (fig. 116). This relaxation is in marked contrast with the contractile reaction of *Alstonia* on the oesophagus (*cf.* fig. 102).

SUMMARY

The results of experiments with indigenous Indian drugs that have been described show that the effect of a particular drug on the different sections of the alimentary tract is not the same but characteristically different. Moreover, while a particular drug may induce a contractile reaction of the organ, another drug might give rise to the opposite reaction of relaxation.

IX.—THE SIGNIFICANCE OF THE PRESENCE OF MANGANESE IN PLANTS

BY

N. C. NAG, M.A., F.I.C.

THE subject of the present paper is the determination of manganese content specially in the *Coniferae* which are indigenous in India as also those introduced from foreign countries; these *Coniferae* are found not only in the hill station of Darjeeling but also in the plains. The influence of habitat, age, as well as inflorescence will form the main subjects of the following investigations.

The importance of manganese as a functionally important element in plants is, perhaps, not generally recognised. The universal occurrence of traces of this substance in animal and plant tissues naturally leads one to conclude that it must have some important physiological function. The substance is found in plants in the seeds, shoots and leaves.¹ In animals it is present in significant amounts in the hair, the liver, the kidneys, the lymphatic glands and in the pancreas.² Blood contains it in fairly constant amounts.³ It is also found in the enzyme-containing tissues and seems to be a potent catalytic agent in the animal and plant kingdoms as it is in the ordinary reactions in a laboratory.

¹ J. S. McHargue, 'The Association of Manganese with Vitamins,' *Jour. Agricul. Research*, vol. 27 (1924), p. 417. 'Further Evidence that Small Quantities of Copper and Manganese are Factors in the Metabolism of Animals' is given in *American Jour. Physiology*, vol. 77 (1926), p. 245.

² J. McCrae, *Jour. South Afric. Chem. Inst.*, vol. 6, p. 18; *Physiolog. Abstr.*, vol. 8 (1924), p. 510.

³ G. Bertrand and F. Medigreceanu, 'Recherches sur le Manganese Normal du Sang,' *Annales de l'Inst. Pasteur*, vol. 26 (1912), p. 1013; 'Recherches sur la Presence du Manganese dans la Serie Animal,' *Annal. Inst. Pasteur*, vol. 27 (1913), p. 282. Quoted by Reiman and Minot, *Jour. Biol. Chem.*, vol. 42 (1920), p. 329.

Bayliss considers it a very important factor in oxidative processes, a peroxidase being probably only an active form of colloidal hydroid of manganese, preserved in active state by the presence of an emulsoid colloid, such as gum or albumin.¹

The necessity of manganese as a nutritive element has received considerable attention during the last two years. Richard² and others³ have indicated the significance of manganese in the reproductive processes of both animals and plants. McCarrison⁴ believes manganese to be necessary for growth of thyroid gland. McHargue went so far as to suggest some connection between the presence of manganese with enzymes and vitamins. McCollum and Orent⁵ have shown that elimination of manganese from an otherwise adequate ration was followed by testicular degeneration in the male rats; in the female the oestrus cycle was not disturbed, but there was deficient lactation.

Clark and Fly⁶ question the universality of the need of manganese by plants. This conclusion is contested by Hopkins,⁷ who advances evidence that manganese is essential and is probably required by all green plants. In this connection I shall adduce experimental results in regard to manganese deficiency which is associated with certain chlorotic leaves.

METHOD OF MANGANESE ESTIMATION

In estimating minute quantities of manganese in plants, the first problem is in the selection of a suitable and reliable

¹ W. M. Bayliss, *Principles of General Physiology* (Longmans, Green & Co., 1927), p. 586.

² M. B. Richard, *Biochem. J.*, vol. 24 (1930), p. 572; *Annual Review of Biochemistry*, vol. 1 (1932), p. 325.

³ Reiman and Minot, Titus and Cave, Meyers and Baird, Titus and Hughes, and others; White House Conference Publication on Growth and Development of the Child. Pt. III.—'Nutrition.'

⁴ R. McCarrison, 'Effect of Manganese on Growth,' *Indian Jour. Med. Research*, vol. 14 (1927), p. 614; *Chem. Abst.*, vol. 21 (1927), p. 2723.

⁵ E. V. McCollum and E. Orent, *J. Maryland Acad. Sci.*, vol. 2 (1931), p. 33; *J. Biol. Chem.*, vol. 92 (1931), p. 651; *Annual Review of Biochemistry*, vol. 1 (1932), p. 325.

⁶ N. A. Clark and C. L. Fly, *Plant Physiology*, vol. 2 (1930), p. 241.

⁷ E. F. Hopkins, *Science*, vol. 74 (1931), p. 551; *Annual Review of Biochemistry*, vol. 1 (1932).

method for quantitative determination under such interfering conditions as the presence of chlorides and of considerable quantities of iron. After a preliminary series of tests, the following procedure was adopted as being not only very suitable but also one which gave concordant results in different sets of experiments.

The freshly gathered parts of plants, such as leaf, bark, and wood, were weighed and burnt in platinum basins or crucibles at as low a temperature as possible. This procedure was found to give better ashing results than the employment of high temperature at the beginning. Certain precautions were found necessary in a few cases where the platinum was found attacked, particularly when the operation was carried out in crucibles with lids on. In these cases, as in the *Araucarias*, there were considerable quantities of chlorine in the plant. Under such circumstances it was better to carry out the ashing in silica vessels or at any rate to transfer the ash first formed in a platinum vessel to a silica one, and fuse the ash with a small quantity of sodium bisulphate, chlorine under these circumstances being got rid of. The fused mass is then extracted with nitric acid. The method generally advocated of fusing the ash with sodium carbonate or sodium nitrate has necessarily to be carried out in a platinum vessel; but this has been found to be risky, since a little platinum carried with the ash interferes with the ammonium persulphate method of manganese determination. The subsequent procedure is the same as described in standard works on quantitative analysis, such as those of Treadwell-Hall and Mitchel.¹

The extract of the ash in nitric acid, after addition of silver nitrate as the catalytic agent, is made up to a known volume with distilled water. Two exactly similar transparent glass test tubes of equal dimensions are next taken; into one is poured 10 c.c. of the nitrate solution and sufficient quantity of ammonium persulphate. The test tube is warmed by placing it in a beaker of water over a flame, the temperature being raised to about 80° C. The permanganate

¹ Treadwell-Hall, *Quantitative Chemical Analysis*, vol. 2; C. A. Mitchel, *Recent Advances in Analytical Chemistry*, vol. 2, p. 158. *

colour develops and the test tube is then cooled. Side by side with this test tube is placed a second one with properly diluted permanganate of known strength so as to exactly match the colour of the solution under examination. The height and diameter of the test tubes with solutions being the same, the amounts of manganese must also be the same when the colours in the two test tubes are exactly matched. It is advisable to add some quantity of acid sodium or potassium phosphate, as it was found that this helps in exact matching, specially when the solutions contain much iron. In a particular case where the manganese is found to correspond with 50 grams in a million grams of leaves, the result is expressed as :

$$\text{Mn in Leaves} = 50 \times 10^{-6}$$

The above refers to fresh leaves which could be examined immediately, the specimen being collected in the neighbourhood of the laboratory. But in other cases the material had to be obtained from a distance ; in such cases the leaves became partially dried up, causing variation in the proportion of the manganese content in normal relation to the weight of the fresh turgid leaf. This defect is obviated by determining the quantity of manganese present in the ash. In these cases the manganese is expressed in percentages. For example, if the amount of ash was one gram and the total quantity of manganese found in it was 0.0045 gram, this would be expressed as :

$$\text{Mn in Ash} = 0.45 \text{ per cent.}$$

meaning that there is 0.45 gram of manganese in 100 grams of the ash.

METHOD OF COMPARING COLOUR

The procedure of matching the colour of the two solutions was modified in the following manner, as it enabled better detection of slight differences in the shade of the two permanganate solutions.

* A right-angled glass prism is mounted so as to have

one of its faces in a vertical plane, the other face being horizontal and facing upwards. The prism and the test tubes are enclosed in a blackened box and so adjusted that when the observer looks vertically downwards he sees the totally reflected parts of the test tubes containing the coloured solutions, the test tubes having been placed just in front of the vertical face of the prism. Diffused light is allowed to pass through a ground glass plate which forms

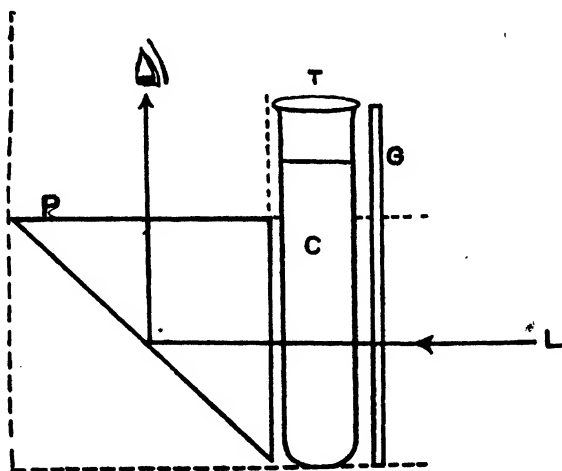


FIG. 117.

T, one of the two test tubes containing the coloured solution c.

G, ground glass plate.

L, direction of the incident light.

P, the right-angled glass prism.

The dotted line represents the blackened casing.

the vertical face of the box, so that the light passing through the two test-tubes, after entering the prism, falls on the inclined plane and gets totally reflected and comes out vertically through the horizontal face to meet the observer's eyes.

A diagrammatic representation of the apparatus is given above (fig. 117).

The results of investigations on the presence of manganese will be described as follows. The effect of change of habitat will be studied in regard to *Pinus longifolia* as well

as other *Coniferae*. The effect of inflorescence on the relative distribution of manganese in different parts of the plant will then be described. And finally the effect of chlorosis in modifying the manganese in certain leaves will be briefly indicated.

PINUS LONGIFOLIA

The effect of change of habitat is studied by observation of the relative quantity of manganese present in the organs of this particular species of plant growing in the hill station of Darjeeling and also in the plains. *Pinus longifolia* is a Himalayan tree, which has been transplanted into hotter and drier climates down in the plains. The quantitative results are given in regard to the manganese content present not only in fresh specimens but also in the ash.

Experiment 1. A young plant of *Pinus longifolia* from Mayapuri, Darjeeling, gave the following results :

Mn, in Leaves	= 33.45×10^{-6}
Mn in Leaf Ash	= 0.33 gram in 100 grams of ash or 0.33 per cent.
Mn in Bark	= 21.45×10^{-6}
Mn in Wood	= 2.23×10^{-6}

Experiment 2. A full-grown tree of *Pinus longifolia* from Darjeeling Botanical Gardens gave the following results :

Mn in Leaves	= 61.60×10^{-6}
Mn in Leaf Ash	= 0.43 per cent.
Mn in Young Wood	= 6.95×10^{-6}
Mn in Old Wood	= 2.0×10^{-6}

From the results given in Experiments 1 and 2, it would appear that the Mn content is, relatively speaking, higher in the leaves than in the bark, being least in the wood. This would seem to indicate that manganese is present in larger quantities in actively growing organs. In regard to the presence of manganese in leaves it is more abundant in a fully grown than in a young tree.

The next series of investigations relate to the effect of change of habitat.

Experiment 3. *A fully grown tree of Pinus longifolia from the Botanical Gardens at Shibpur, near Calcutta, gave manganese contents which are considerably less than in the hill specimens :*

Mn in Leaves	= 21.00×10^{-6}
Mn in Leaf Ash	= 0.20 per cent.

Experiment 4. *Pinus longifolia from Benares Hindu University Gardens.*—The tree was 16 years old and about 8 feet in height, and gave the following results :

Mn in Leaves	= 16.00×10^{-6}
Mn in Leaf Ash	= 0.11 per cent.

In recapitulating the results, the influence of habitat is demonstrated by the fact that the manganese content is comparatively less in trees grown in the plains ; the results obtained in Calcutta (Experiment 3) fully support this. Further, the Benares tree grown in a different locality at a distance of about 400 miles from Calcutta exhibits the smallest amount (Experiment 4).

ARAUCARIA

I next give results obtained with some other Conifers, namely, *Araucaria*, the specimens having been obtained from different localities. Some of the specimens belong to the species *A. Cookii*, others to those of *A. Cunninghamii* and *A. Bidwellii*. The results obtained will be found to demonstrate the effect of habitat on the manganese content.

ARAUCARIA COOKII

Experiment 5. *A. Cookii grown at Darjeeling* gave the following results, which may be taken as the average of a large number of determinations :

Mn in Leaves	= 46.0×10^{-6}
Mn in Leaf Ash	= 0.21 per cent.

Experiment 6. *A. Cookii* from the Bose Institute Gardens, Calcutta, gave the following results :

Mn in Leaves	= 32.55×10^{-6}
Mn in Leaf Ash	= 0.17 per cent.

Experiment 7. *A. Cookii* from Shibpur Botanical Gardens, Calcutta, gave the following results :

Mn in Leaves	= 21.88×10^{-6}
Mn in Leaf Ash	= 0.04 per cent.

From Experiments 5, 6 and 7 it is clear that the manganese content undergoes a marked diminution when specimens of this particular *Araucaria* are grown in the plains instead of at higher altitudes.

ARAUCARIA CUNNINGHAMII

Parallel variation in manganese content was also found in the case of *Araucaria Cunninghamii*.

Experiment 8. *A. Cunninghamii* from Darjeeling Botanical Gardens gave the following results :

Mn in Leaves	= 82.5×10^{-6}
Mn in Leaf Ash	= 0.42 per cent.

Experiment 9. *A. Cunninghamii* from Bose Institute Gardens, Calcutta. The results are as follows :

Mn in Leaves	= 55.13×10^{-6}
Mn in Leaf Ash	= 0.28 per cent.

Experiment 10. *A. Cunninghamii* from Shibpur Botanical Gardens, Calcutta. The quantitative results are given below :

Mn in Leaves	= 46.35×10^{-6}
Mn in Leaf Ash	= 0.15 per cent.

Here also the presence of manganese in the plants grown in the plains is considerably lower than in the hills.

ARAUCARIA BIDWELLII

This is another species of *Araucaria* which has been introduced into India from a foreign country.

Experiment 11. *A. Bidwellii* from the Darjeeling Botanical Gardens gave the following results :

Mn in Leaves	=	198.0×10^{-6}
Mn in Leaf Ash	=	0.80 per cent.

Experiment 12. *A. Bidwellii* from Shibpur Botanical Gardens, Calcutta.

Mn in Leaves	=	46.33×10^{-6}
Mn in Leaf Ash	=	0.185 per cent.

In these cases also the manganese content in trees grown in the plains is considerably lower than in those grown in the hills.

THUJA ORIENTALIS

Thuja is found both in the hills and in the plains of India.

Experiment 13. *Thuja orientalis* at Darjeeling gave the following results :

Mn in Leaves	=	148.8×10^{-6}
Mn in Leaf Ash	=	1.06 per cent.

Experiment 14. *Thuja orientalis* from Shibpur Botanical Gardens, Calcutta :

Mn in Leaves	=	38.16×10^{-6}
Mn in Leaf Ash	=	0.11 per cent.

Experiment 15. *Thuja orientalis* from Benares Hindu University Gardens :

Mn in Leaves	=	22.0×10^{-6}
Mn in Leaf Ash	=	0.05 per cent.

It will be noticed that while the Darjeeling specimen gives a fairly high manganese content, those in the plains contain a considerably less amount. Further, while the Shibpur specimen gives a low value, that of Benares contains the least amount.

What is the significance of the presence of relatively larger amounts of manganese in the Conifers grown in the

hills as compared with those grown in the plains? Certain results given in detail in Experiment 2 seemed to indicate that the more abundant presence of manganese connotes greater vital activity. On this assumption it would appear that a hill station such as Darjeeling is likely to be a more suitable place for growth of Conifers like *Thuja* than are the plains.

PODOCARPUS

Certain species of *Coniferae* find, however, a more suitable habitat in the plains than when they are transferred to the hills. This is specially the case with different species of *Podocarpus*, which are known to be plants naturally growing in the plains of the Indian peninsula.

PODOCARPUS NERIFOLIUS

Experiment 16. *P. nerifolius* from Darjeeling Botanical Gardens was examined and gave the following results:

Mn in Leaves	= 16.5×10^{-6}
Mn in Leaf Ash	= 0.09 per cent.

Experiment 17. *P. nerifolius* from Shibpur Botanical Gardens, Calcutta:

Mn in Leaves	= 34.41×10^{-6}
Mn in Leaf Ash	= 0.11 per cent.

PODOCARPUS MACROPHYLLUS

Experiment 18. *P. macrophyllus* from Darjeeling Botanical Gardens:

Mn in Leaves	= 16.5×10^{-6}
Mn in Leaf Ash	= 0.09 per cent.

Experiment 19. *P. macrophyllus* from Shibpur Botanical Gardens, Calcutta:

Mn in Leaves	= 32.82×10^{-6}
Mn in Leaf Ash	= 0.15 per cent.

In the case of *Pinus longifolia* already described, the results have been found to be the very opposite to those in *Podocarpus*. The manganese content in the leaves of hill specimens of *Pinus longifolia* is thus relatively higher than in those of the plains; from this it was inferred that the vital activity of the hill specimens of *P. longifolia* was relatively higher. Following the same course of reasoning, the opposite results in *Podocarpus* would lead to the conclusion that the hill specimens were physiologically less active than those of the plains. The plains would therefore appear to be more suitable for the growth of *Podocarpus* than are the hills.

EFFECT OF INFLORESCENCE

I next deal with the question whether inflorescence has any effect in modifying the manganese content. The following investigations were carried out with certain species of *Coniferae* growing at Darjeeling, the manganese contents of which were exactly determined before and after inflorescence. The typical examples for this study were obtained from *Abies balsamia*, *Picea morinda* and *Cryptomeria japonica*.

ABIES BALSAMIA

In the genus *Abies* (Silver Fir) the manganese content has elsewhere been found to be relatively high.¹ The leaves of numerous specimens of *Abies* which I examined showed a general characteristic that leaves from older trees of a particular species contained more manganese than those from the younger specimens. The following gives typical results of manganese content of leaves in *Abies balsamia*, before and at the time of flowering.

Experiment 20. The particular specimen of *Abies balsamia* growing at Mayapuri, Darjeeling, is a small tree about two feet in height. The manganese content of the leaves was determined before inflorescence in the month of October and found to be :

$$\text{Mn in Leaves} = 155.0 \times 10^{-6}$$

¹ J. Goessl, *Über das Vorkommen des Mangan in der Pflanze u.s.w.* Beihoft. Z. Bot. Zbt., 1905.

Experiment 21. *Effect of female inflorescence.*—The particular tree referred to in the last experiment bore later, in April, female flowers and small cones. The leaves above the cones were examined to find whether there was now any difference in their manganese content compared with their normal distribution before flowering. The results are given below :

Mn in Leaves during female inflorescence = 430.5×10^{-6}

From the above two experiments it will be seen that during the period of female inflorescence the manganese content of the leaves had increased from the normal 155.0×10^{-6} to 430.5×10^{-6} . The increase of manganese thus appears to be in some way connected with the reproductive process associated with the formation of cones. These latter containing the ovules were then examined in the next experiment.

Experiment 22. *Examination of manganese content in the cone.*—It is to be borne in mind that the cones consist not only of ovules but numerous woody scales which protect them. In spite of the large quantity of the comparatively indifferent woody tissue the manganese content of the cone, taken as a whole, was found to be fairly high.

Reference has already been made to the fact that the leaves have their manganese content greatly increased during the female inflorescence. These leaves in the flowering branches are contiguous to the cones, in which latter the presence of large quantities of manganese would appear to be necessary. The large quantity of manganese stored in the neighbouring leaves might easily serve as the source of supply.

PICEA MORINDA

Results similar to the above were observed with a specimen of *Picea morinda* tree growing in the *Botanical Gardens, Darjeeling*.

Experiment 23. Some branches of the tree bore cones, while there were others which did not. Quantitative determination was made of the manganese content in the

leaves from the cone-bearing branches, as also in the leaves from branches which did not bear any cones. It was found that while the leaves of cone-bearing branches contained a very large quantity of manganese, the manganese content of leaves from branches without cones was decidedly less.

CRYPTOMERIA JAPONICA

Cryptomeria japonica was introduced into India from further East nearly a century ago, and grown extensively from Kurseong to Darjeeling. I had opportunity of examining various specimens from different localities of Darjeeling.¹

Experiment 24. *Cryptomeria japonica* from Mayapuri Hillside.—I examined a large number of young specimens of the plant in which there was no fructification. The average result is given below :

$$\text{Mn in Leaves} = 16.0 \times 10^{-6}$$

Experiment 25. The following results relate to the manganese content of other *specimens growing on the hillside* in which cones were being formed :

$$\begin{aligned}\text{Mn in Leaves after flowering} &= 26.4 \times 10^{-6} \\ \text{Mn in Green Cones} &= 12.49 \times 10^{-6}\end{aligned}$$

Experiment 26. *Cryptomeria japonica* from Darjeeling Botanical Gardens.—The following results relate to the manganese content in a specimen growing in a different locality, the tree being full of cones :

$$\begin{aligned}\text{Mn in Leaves} &= 24.75 \times 10^{-6} \\ \text{Mn in Cones} &= 19.80 \times 10^{-6}\end{aligned}$$

In reviewing the results obtained with a large number of different species of *Coniferae*, as given in detail in the fore-

¹ I take this opportunity of thanking Mr. B. Ghose of Town End, Darjeeling, for some of the specimens with which I worked. My thanks are also due to Mr. S. Bose, Curator, Darjeeling Botanical Gardens, and to Mr. N. Mitter, Curator, Shibpur Botanical Gardens, Calcutta, for some of the other specimens.

going experiments, we find that the manganese content of the leaves undergoes a marked modification during female inflorescence. It is the leaves in branches containing the cones that exhibit a very great increase in their manganese content. The cones also are found to contain an excess of manganese which is probably drawn from the stored supply in the leaves. It should be stated in this connection that there is no noticeable increase of manganese content in leaves of the *Coniferae* examined when they bore only male flowers. The increase in manganese content in the leaves is, as already stated, very marked during the production of female flowers leading to the formation of cones.

CHLOROTIC LEAVES AND THEIR MANGANESE CONTENT

The following investigations were undertaken to find out whether there is any marked variation in the manganese content in normal green and in chlorotic leaves. Certain plants were found in which a number of branches bore vigorous green leaves, while those borne by other branches were in a chlorotic condition. In order to subject the matter to quantitative tests, a large number of green leaves were examined and their average manganese content determined. The same procedure was followed in regard to the average manganese content in the chlorotic leaves.

The following investigations were carried out with specimens of plants which were growing in the *Gardens of the Bose Institute, Calcutta*. The first of these relates to *Mimosa spegazzinii* and the second to *Desmodium gyrans*.

Experiment 27. *Determination of difference of manganese content in green and chlorotic leaves of Mimosa spegazzinii*. For this a series of determinations were made with leaves of the tree-like *Mimosa*, every one of whose leaves was green and healthy, as also with those from other specimens which were partially chlorotic—that is to say, in which certain branches bore healthy green leaves while others bore chlorotic leaves. The following gives the average quantitative results in the three cases :

- (a) Mn content in *Green* Leaves of a Healthy Tree = 68.0×10^{-6}
- (b) Mn content in *Green* Leaves of a partially Chlorotic Tree . . . = 67.5×10^{-6}
- (c) Mn content in *Chlorotic* Leaves of a partially Chlorotic Tree . . = 24.0×10^{-6}

It will be noted that while the manganese content of green leaves from a healthy or a partially chlorotic tree is practically the same, that from the chlorotic branch is markedly less, being reduced to nearly a third of the normal amount.

A similar investigation was next carried out on the relative proportion of manganese present in healthy green and chlorotic leaves of *Desmodium gyrans*.

Experiment 28. *Determination of difference of manganese content in green and chlorotic leaves of Desmodium gyrans.*—The following is a statement of average results :

Mn content in normal *Green* Leaves = 57.5×10^{-6}

Mn content in *Chlorotic* Leaves = 18.5×10^{-6}

The above results would indicate that for the proper functioning of normal activity of green leaves, one of the important factors, though not the only one, is the presence of a sufficient quantity of manganese.

SUMMARY

A large number of experiments were carried out with numerous species of *Coniferae* grown in the hills at Darjeeling and in the plains at Calcutta, Shibpur and Benares. The amount of manganese present in the leaves of different specimens varied characteristically according to the species of the plant. Moreover, in the same species the manganese content underwent a change according to its habitat. The Himalayan *Coniferae*, whose natural habitat is at a high level, exhibit a diminution of the manganese content when they are grown in the plains. The peninsular *Coniferae* such as *Podocarpus* which grow naturally at lower altitudes

show, on the other hand, a higher manganese content in the plains than when grown in the hills.

The manganese content in the leaves of *Coniferae* undergoes a marked increase during the production of female flowers ; this increase of manganese content in the leaves of flowering branches is probably the source for the supply of the relatively large amount of manganese found in the cones. *Coniferae* which give rise to male inflorescence only do not show any noticeable increase of manganese content in their leaves.

In healthy green leaves of *Mimosa spegazzinii* and of *Desmodium gyrans* the manganese content is much higher than what is found in chlorotic leaves of the same plants. This seems to indicate that a sufficient quantity of manganese is one of the factors for proper functioning of the green leaves.

I gratefully acknowledge the encouragement that I received from Sir J. C. Bose during the course of these investigations. His criticism and suggestions have been most helpful in the present work.

X.—AN ORGANIC PRINCIPLE IN *EUPATORIUM* *AYAPANA*, VENT.

BY

N. C. NAG, M.A., F.I.C., AND K. N. BOSE, B.Sc.

In a previous communication¹ on the chemical composition of *Eupatorium Ayapana* we dealt with the principal inorganic constituents found in the watery extract as well as in the ashes of the plant. It was there stated that we had been able to isolate a crystalline compound with a definite melting-point. In the present investigation we shall deal with the method for extraction of the pure compound and the determination of its composition and the preparation of some of its derivatives. Certain allied facts will also be described regarding the properties of the compound after its isolation from the plant.

METHOD FOR ISOLATION

Extracts are made from thoroughly dried and powdered leaves in water kept boiling on a hot plate. The hot solution is strained through a cloth and evaporated down to a syrupy consistency in a porcelain basin on a water bath. The syrupy liquid is then transferred to a glass-stoppered bottle and shaken up from time to time with about 5 times its volume of rectified spirit (about 91 per cent. alcohol). The bottle is then left in a warm place overnight. Most of the mineral matter will have by this time been thrown down as a sediment below a clear supernatant liquid. The liquid is then filtered into a distilling flask and the alcohol is removed and recovered by distillation. The residual liquor in the

¹ N. C. Nag and K. N. Bose, *Trans. Bose Inst.*, Vol. vi (1930-31).

distilling flask is then transferred to a large-sized glass-stoppered separating funnel, and shaken up with petroleum ether or benzol. The solvent will then have taken up the organic compound, which is far more soluble in organic solvents than even in boiling water. The separated solvent with the substance in solution is then evaporated down; this leaves a crystalline residue in a matrix of chlorophyll and of some yellowish gummy matter. The residue is then boiled with water on a water bath and *filtered hot* through a hot jacketed funnel. The boiling water dissolves and carries down in solution the crystalline substance freed from the impurities. On cooling, the crystals become reformed and come out of the watery extract in which the substance is sparingly soluble. The crystals filtered out from the cold water require further recrystallisation; the process of dissolving in boiling water and recrystallising in cold is repeated three times in succession for securing the purest product. The crystals are air-dried first and then placed in an air oven at a temperature of 60° C. to 80° C. The crystals are finally kept in a vacuum desiccator.

Fifty grams of dry powdered leaves (with youngest portion of twigs) gave by the above procedure 0.095 gram of the crystalline substance; the yield, therefore, is about 0.2 per cent. of the dry matter.

COMPOSITION AND MOLECULAR FORMULA

A preliminary qualitative examination of the crystalline substance showed it to be composed of carbon, hydrogen and oxygen. The substance on combustion left no residue nor did it answer to tests for nitrogen, sulphur, halogens or phosphorus.

A number of combustion analyses were carried out, and gave the following results:

	I	II	III
Percentage of Carbon . . .	67.00	66.55	66.56
„ „ Hydrogen . . .	4.00	4.31	4.21
„ „ Oxygen (by diff.)	29.00	29.14	29.23

The determination of molecular weight carried out by the

method of rise of the boiling point of (a) acetone, and of (b) alcohol gave the following results :

(a) 226

(b) 225

From the above, the probable molecular formula would be $C_{12}H_{10}O_4$, corresponding to the molecular weight 218. The theoretical percentage of carbon would then be 66.05, and of hydrogen 4.53.

The above conclusion is fully confirmed by the recent Micro-chemical Method of Rast.¹ This we found to give 220 as the molecular weight.

PROPERTIES

The crystalline substance is almost colourless; the crystalline form is shown in the accompanying reproduction from a micro-photograph (fig. 118).



FIG. 118. Crystals from *Eupatorium Ayapana*.

The melting-point was determined in specimens of crystals prepared from plants collected from different sources. The melting-point was found to be fairly constant, lying between 110° C. and 112° C. As the temperature is raised, the colour of the liquid deepens to yellow and remains still undecomposed even at the high temperature of 210° C.

¹ Pregl-Fyfe, *Quantitative Organic Micro-Analysis* (1930), p. 217; *Ber.* vol. 55 (1922), pp. 1051, 3727.

The solubility of the substance in water at 26° C. was found to be 0.05 gram in 100 c.c. of water. It is readily soluble in acetone and slightly less soluble in ethyl ether, petroleum ether and benzol. Addition of a drop of concentrated sulphuric acid to the crystalline substance gives a violet colouration. It dissolves as a yellow solution in concentrated nitric acid, from which a yellow product separates out on dilution, indicative of the substance being of an aromatic nature. Further investigations on this will be described later. Bromine gives a tetrabromo-compound of a slightly yellow colour, by absorption of bromine when shaken up in cold water.

In warm concentrated caustic alkali, the substance dissolves to a yellow solution. On cooling the substance still remains in solution. On acidification with dilute hydrochloric acid, a white substance is precipitated which is soluble in hot water and which crystallises out on cooling. These particular crystals were found to melt at 111° C.

SUMMARY

A new organic compound has been isolated from the extract of *Eupatorium Ayapana*. A crystalline substance with definite melting-point at or about 111° C. has thus been obtained by repeated crystallisation from special solvents.

The purified product was subjected to combustion analysis and the percentages of carbon, hydrogen and oxygen were determined. Molecular weight of the substance was determined by boiling-point method in acetone and in alcohol, and also confirmed by micro-cryoscopic method of Rast in camphor.

From the results thus obtained, the molecular formula of the compound is found to be $C_{12}H_{10}O_4$.

The substance seems to be of an aromatic nature. Description is given of its characteristic properties and its reaction under the action of different reagents.

We gratefully acknowledge our indebtedness to Sir J. C. Bose for his kind interest and encouragement throughout the investigation.

XI.—CHEMICAL EXAMINATION OF OILS FROM LEGUMINOUS PULSES

BY

H. N. BANERJEE, M.Sc.

THE results of investigation on the composition and properties of oils from *Phaseolus radiatus* (var. *shona moog*) and *Cicer arietinum* (*chhola*) have been given in previous communications.¹ The subjects of the present Paper relate to chemical examination of oils from certain other varieties of *Phaseolus radiatus* and of *Cicer arietinum*. These will be classified under headings (3) and (4).

In regard to *Phaseolus radiatus*, there are several varieties of it; the properties of the oil from *shona moog* have already been described. In the present Paper I shall deal with those of *green moog*.

(3) *PHASEOLUS RADIATUS* (var. *GREEN MOOG*)

The method of extraction of the oil was the same as described in the previous communications. Finely powdered *green moog* was treated with dry ether by the Soxhlet Extraction Method, and the following constants determined :

PHYSICAL AND CHEMICAL CONSTANTS OF THE OIL (*GREEN MOOG*)

Oil yield	Refractive Index at 31° C.	Saponification value	Iodine value	Fatty acid	Unsaponifiable matter
0.5 %	1.4770	171.2	111.9	80.0 %	12.3 %

¹ N. C. Nag, H. N. Banerjee and K. Bose, *Proc. Sixteenth Ind. Sc. Congress*; H. N. Banerjee, *Trans. Bose Inst.*, Vol. vii (1931-32).

FREE FATTY ACID CONSTANTS

Iodine value	Neutralisation value	Mean molecular weight
117.04	196.35	285.7

SOLID AND LIQUID ACIDS

Solid acid				Liquid acid		
Yield %	Melting point	Solidifying point	Iodine value	Yield %	Refractive index	Iodine value
47.9	52° C.	48° C.	58.7	52.1	1.4680 at 35° C.	164

CONSTITUENTS OF THE LIQUID UNSATURATED ACID MIXTURE

Separation and estimation of the constituents of the liquid unsaturated acid mixture were effected by the bromination method of Eibner and Muggenthaler.

The different bromides	Yield	Calculated acid	Percentage of individual acid
Oleic Dibromide	1.047	0.6682	46.2 % Oleic Acid
Linolic Tetra-bromide	1.432	0.6682	46.2 % Linolic Acid
Linolenic Hexa-bromide	0.30	0.11	7.6 % Linolenic Acid

UNSAAPONIFIABLE MATTER

The unsaponifiable matter, which was 12.3 per cent. of the oil, gave strong Liebermann-Burchard reaction as well as Salkowski reaction for sterols. After several successive evaporations from 98 per cent. alcohol, the substance was obtained in powdery form with yellow pigment. This began to soften at 120° C. and became fully melted at 125° C. The substance was redissolved in *methyle alcohol* and after reprecipitation it was obtained as white powder free from colouring matter. The melting-point of this powder was, however, not sharp, lying between 125° C. and 130° C. Even after a number of reprecipitations the melting-point of the powder still remained somewhat indefinite. The substance was, therefore, acetylated by acetic anhydride treatment. The acetate obtained melted between 120° C. and 123° C. The above facts showed that the sterol obtained was still a mixture. The substance was then subjected to the digitonin method of purification.

CRYSTALLINE STEROL BY DIGITONIN METHOD

The digitonin solution of 0.5 per cent. in 95 per cent. alcohol was added to the solution of the mixed sterols in 95 per cent. alcohol, and the mixture was left over night; this gave a copious white precipitate of sterol digitonide. The precipitate was filtered, washed with 95 per cent. alcohol and boiled with xylene. The xylene solution containing the sterol was filtered off from the separated digitonin, which remained as an insoluble residue. The xylene solution was then evaporated to dryness and the sterol was crystallised from a solution in 95 per cent. alcohol.

The crystalline needles obtained by this procedure were found to melt at a definite point which was slightly above 132° C., but below 133° C.

CAROTENE

The yellow colouring matter present in the unsaponifiable portion of the oil was afterwards identified as carotene.

(4) *CICER ARIETINUM* (*KABULI CHHOLA*)

The method for extraction of the oil from *Kabuli chhola* was the same as in the other cases of extraction by the Ether Soxhlet process. The determination of the various constants was made by the usual methods previously described. In a few cases additional descriptions are given of recent modifications that have been found to be more suitable.

In order to bring out certain characteristic differences in the oils from the two varieties of *Cicer arietinum*, namely, A. *Kabuli chhola* and B. *Common chhola*, their physical and chemical constants are given in the same table for facility of comparison.

PHYSICAL AND CHEMICAL CONSTANTS OF THE OILS

A. *Kabuli chhola* ; B. *Common chhola*.

Oil yield	Saponification value	Acetyl value	Acid value	Iodine value	% of fatty acid
A. 4 %	185.3	2.3	9.85	72.44 (Hanus)	87.42
B. 4.2 %	184.2	—	4.2	129-130	92.6

As regards the difference between the two oils the comparatively low iodine value of *Kabuli chhola* oil is specially noticeable. Associated with this is the relatively non-drying quality of the same oil.

The refractive index at 23.5° C. for *Kabuli chhola* oil was found to be 1.4775, the quantity of unsaponifiable matter was 3.4 per cent., while the respective values for *common variety of chhola* oil were 1.4780 at 29° C. and 1.5 per cent. It is to be noted that the quantity of unsaponifiable matter in the *Kabuli* variety is much higher than in the ordinary variety. Vitamin content as determined

by SbCl_3 test and Lovibond Tintometer, is also much higher. The refractive index of the oil from the common variety is, as already stated, slightly higher, being possibly due to the presence in it of higher unsaturated acids.

MIXED FATTY ACIDS

The *Kabuli chhola* oil was saponified according to the modified process of Malfatti.¹ The unsaponified matter was completely removed and the free fatty acids liberated by the usual process. After the usual drying in vacuum desiccator the constants were determined.

Refractive index	Iodine value	Neutralisation value	Mean molecular weight
1.4695 at 27°C.	85.11	186.2	300

SEPARATION OF THE LIQUID UNSATURATED FROM THE SOLID SATURATED ACIDS

The separation was carried out according to Twitchell Method. A weighed quantity of the mixed fatty acids was dissolved in a known volume of 95 per cent. alcohol. In another vessel was dissolved crystalline lead acetate, 1.5 grms. per 50 c.c. of 95 per cent. alcohol. Both the solutions were heated to boiling, the lead acetate solution poured into the fatty acid solution and the mixture left aside for 24 hours and then filtered. The insoluble lead salt of the solid saturated acid was again dissolved in 100 c.c. of 95 per cent. alcohol, containing 0.5 gram. of glacial acetic acid. After cooling the filtered lead salt was washed thoroughly with 95 per cent. alcohol. The solid saturated fatty acid was obtained by decomposition of the solid lead salt by the action of dilute nitric acid.

¹ Malfatti, *Chem. Abstracts*, vol. 6 (1912), p. 200; *Vegetable Oils and Fats*, by Jamieson (1932), p. 339.

The alcoholic mother liquors from the solid salt were mixed together and the bulk of the alcohol distilled off under reduced pressure over a water bath ; from the clear solution left, the liquid unsaturated acid was obtained by decomposition with excess of dilute hydrochloric acid.

The various constants were then determined, as also the relative proportions of the solid and liquid acids in the fatty acid mixture.

SOLID AND LIQUID ACIDS

Solid acid		Liquid acid		
Yield	Iodine value	Yield	Refractive index	Iodine value
10 %	4.82	90 %	1.4670 at 24° C.	89.8

IDENTIFICATION OF THE LIQUID UNSATURATED ACID BY BROMINATION METHOD

The liquid unsaturated acid obtained was dissolved in dry ether cooled down to ten degrees below zero (-10°C.) and 1 c.c. bromine added drop by drop. The mixture was kept aside in the freezing bath for half an hour, the contents having been kept stoppered all the time. There was absolutely no separation of solid bromo-derivative. This indicated the absence of any linolenic acid, there being no hexa-bromide separated. The ethereal solution was next repeatedly washed with sodium thiosulphate solution to get rid of free bromine ; it was then washed thoroughly with distilled water to remove any adhering hypo. After evaporation of the ether, a deep brown oil only was left behind ; this indicated the presence of oleic di-bromide ; since there was no solid left, there could be no tetra-bromide.

In confirmation of the above, the bromine content of the brown liquid was determined and found to be 36.4 per cent. Theoretically oleic di-bromide, $\text{C}_{18}\text{H}_{34}\text{Br}_2\text{O}_2$, corresponds to 36.18 per cent. of Br.

From the iodine value previously determined, as also from the bromo-derivative now obtained, it is evident that the liquid unsaturated acid in *Kabuli chhola oil* is only oleic acid, since no higher unsaturated acid could be detected. In my previous Paper it was shown that the oil from the *common variety of chhola* did not contain only oleic acid, in contrast with the above, but also others, namely, 59.36 per cent. linolic and 2.23 per cent. linolenic acids.

The oleic acid obtained from *Kabuli chhola oil* on standing becomes bleached and perfectly white and a 'tallowy' odour and flavour characteristic of oxidised fat become developed¹; after this it becomes partially insoluble in petroleum ether.²

STEROL FROM UNSAPONIFIABLE MATTER

The oil was saponified, the soap dissolved in water and the unsaponifiable matter recovered by extraction with ether. After the usual processes of washing thoroughly, an orange-coloured product was obtained by evaporating away the ether. This was dissolved in the minimum quantity of boiling 98 per cent. alcohol. On cooling, crystals of sterol were obtained. These after double crystallisation from boiling alcohol were obtained as tufts of beautiful crystals, the melting-point of which was between 134° C. and 135° C.

CAROTINOIDS

The alcoholic mother liquor obtained after separation of the sterol crystals was evaporated and a deeply orange-coloured product was obtained. This answered to tests for carotinoids.

PHYTO-STEROL ACETATE

The sterol crystals were boiled in a small conical flask with their own weight of acetic anhydride under a reflux condenser for one hour. After the completion of the reaction, the excess of acetic anhydride was evaporated away

¹ C. H. Lea, *J.S.C.I.*, vol. 52 (1933), 10 T, *Special Food Number*.

² Fahrion, *Zeits. f. ange. Chem.* (1909), p. 2093; (1910), pp. 722, 1108. Lewkowitsch, *Oils and Fats*, Pt. i, p. 593.

on a water bath. The acetate was crystallised from its solution in boiling 95 per cent. alcohol. After two such successive crystallisations from alcohol, the pure sterol acetate was obtained, as indicated by its definite melting-point at 124°C .

BENZOYL DERIVATIVE OF THE STEROL

A quantity of the sterol was benzoylated according to the Schotten-Baumann process. The pure crystalline sterol was kept suspended in water, and benzoyl chloride and a 10 per cent. caustic soda solution were added alternately in small quantities. Thus after the addition of the benzoyl chloride the mixture is made alkaline by the addition of caustic soda and the tube is shaken up until the smell of benzoyl chloride is completely removed, the mixture being rendered permanently alkaline. The sterol benzoate which separated out was finally filtered, washed with water and crystallised from 95 per cent. alcohol.

The melting-point of the benzoyl derivative was now found to be definitely at 130°C .

LITHIUM STEROL COMPOUND

For the preparation of this compound the Zucker Method¹ was followed; the pure sterol was dissolved in pyridine and a hot saturated solution of lithium chloride in pyridine was added to it. The excess of pyridine was then evaporated away on a water bath. The residue was dissolved in 95 per cent. alcohol. On standing, needle-shaped crystals of lithium sterol compound separated out. The melting-point of this compound was found to be very definite, lying between 131°C . and 132°C .

DIGITONIN STEROL COMPOUND

An alcoholic solution of the sterol was treated with an equal volume of a 0.5 per cent. alcoholic solution of digitonin,

¹ Zucker, *Pharm. Weekblad*, vol. 54 (1917), p. 101; *Vegetable Oils and Fats*, by G. S. Jamieson (1932), p. 312.

when a voluminous precipitate of the digitonin sterol compound was produced, from which the pure sterol could be easily obtained.

Most of the vegetable oils are liable to become rancid by exposure to air and light. The oil from *Kabuli chhola* has in this respect certain distinctive characteristics. Its keeping quality was therefore tested in the following manner.

KEEPING QUALITY AND RANCIDITY DETERMINATION

In order to follow the changes induced in the oil by exposure to air and light, two portions of the freshly prepared oil were placed, one in a vacuum desiccator and another in an ordinary desiccator containing air. Observations were then taken at intervals to find whether any objectionable odour or rancidity became developed. The observations were continued for a period of two months.

The view that auto-oxidation is the primary cause of the onset of rancidity is now generally accepted. The first stage in this spontaneous oxidation of fat is the probable formation of peroxides at the ethenoid linkages of the unsaturated acids. For the detection of the earliest stages in the progressive oxidation, samples from the two portions of the oil in the desiccators were taken at intervals and separately tested, following the Lea Method.¹

Test for Peroxide Oxygen.—One gram of oil was taken in a test-tube and to it were added one gram of finely powdered potassium iodide and 5 c.c. of a glacial acetic acid-chloroform mixture in the proportion of 2 : 1 by volume. The tube was filled with dry CO₂ gas and plugged with loose cotton. The tube under constant shaking was heated over a small flame. After removal of the cotton plug a rubber stopper was inserted, and the tube vigorously shaken and then cooled. Freshly prepared starch paste was then added. The absence of any blue colouration indicated that there was no formation of peroxide. Further tests were carried out in regard to rancidity.

¹ C. H. Lea, *Proc. Roy. Soc., B*, vol. 108 (1931), p. 175; *J.S.C.I.*, vol. 50 (1931), p. 215 T; vol. 52 (1933), p. 9 T, *Special Food Number*.

The Kreis Test for Rancidity (Lea Modification).—One gram of oil was dissolved in 2 c.c. of benzene, and to this was added 1 c.c. of concentrated hydrochloric acid. A fresh rubber stopper was inserted and the tube was vigorously shaken for about a minute, after which 1 c.c. of a 0.1 per cent. solution of phloroglucinol in ether was added and the tube again shaken for another minute. On being centrifuged, two distinct layers of solution were obtained in the test-tube. The test of rancidity is the development of pink colour in the aqueous solution. With *Kabuli chhola oil* there was no such colouration, proving that this particular oil was free from rancidity. It will presently be shown that the reaction is very different in *ordinary chhola oil* as well as in certain other oils. Another test of rancidity is described below.

The Schiff Reaction.—One gram of oil was weighed into a test-tube, 1 c.c. of chloroform was added and the mixture was warmed and shaken. The tube was then cooled and 1 c.c. of a 0.5 per cent. fuchsin sulphurous acid reagent was added. After closing the open end of the test-tube by means of a clean rubber stopper, the mixture was shaken for one minute and allowed to stand in a dark cupboard for 10 minutes. The emulsion did *not* turn *violet*, indicating absence of rancidity.

All the three above tests indicate the absence of rancidity in the *Kabuli chhola oil* in contrast with the production of rancidity in other oils such as *common chhola oil*, olive oil, and cod liver oil, as indicated by the positive reactions in the three latter with definite indications of rancidity and peroxide formation.

TEST FOR VITAMINS

There is a very important and significant reaction in the *Kabuli chhola oil* in regard to the possible presence in it of both A and D vitamins. Such vitamins are found pre-eminently in Cod Liver Oil, the chemical test for the detection of which is the blue-green colouration which develops after the addition of SbCl_3 in chloroform solution. Is there any

possibility of the presence of such vitamins in the particular vegetable oil ?

I give a short account of the tests that I applied in answer to the particular inquiry.

TEST FOR VITAMIN A

Two solutions were made, one a 20 per cent. solution of the *Kabuli chhola oil* in chloroform, the other a 30 per cent. SbCl_3 solution in chloroform. Ten volumes of SbCl_3 solution were added to one volume of the *Kabuli chhola oil* solution. The blue colour, which appeared immediately, soon changed to green. This is taken as a characteristic reaction of Vitamin A.

TESTS FOR VITAMIN D

For this the following three independent tests, (a), (b), and (c), were employed.

(a) Three c.c. of the oil were added to 3 c.c. of a mixture of one volume of concentrated hydrochloric acid with 15 volumes of freshly distilled aniline. The contents were heated to boiling, with constant shaking for one and a half minutes. The yellow emulsion turned first into green and then into red. Upon separation into two layers, it was the lower layer that developed the red colour, which became deeper on standing.

(b) Five c.c. of the oil mixed with 10 c.c. of chloroform and 1 c.c. of acetic acid were shaken with 2.5 c.c. of a 10 per cent. solution of bromine in chloroform. A deep green colour was developed.

(c) A small quantity of the oil was mixed with a few drops of concentrated sulphuric acid and shaken with chloroform ; this resulted in the development of red, which soon changed to violet colour.

Parallel experiments which I carried out with *Cod Liver Oil* known to contain Vitamins A and D gave results which are exactly similar to those with *Kabuli chhola oil*.

The results which have been given in detail indicate the

probable presence of the two vitamins in the particular vegetable oil, and further investigations on the subject therefore promise to be of some interest.

I take this opportunity of expressing my gratitude to Sir J. C. Bose for constant encouragement received from him, and to Professor N. C. Nag for guidance and valuable suggestions.

XII.—THE RACIAL AFFINITIES OF THE MUNDAS

BY

PROVASH CHANDRA BASU, M.B., M.Sc.

(Premchand Roychand Scholar.)

THE hills of Chota Nagpur are inhabited by a group of aboriginal people speaking both the Austric and the Dravidian forms of speech. In discussing their racial affinities Risley was of opinion that linguistic differences merely concealed their basic somatic unity. He considered that the Dravidian- and the Austric-speaking groups really belonged to a single racial stock which with the primitive inhabitants of the Central Indian highlands and the hills of Orissa formed an ancient and large ethnic block that had been broken up into separate groups by later movements of people.¹ How far Risley's contention is justified by facts is one of the fundamental problems in connection with the racial composition of the aborigines of this country, nay of Indian anthropology in general, as the latter has undoubtedly entered into the composition of the lower stratum of the Indian population. As, however, there is a constant pressure on these peoples from their more advanced neighbours, it is essential that investigations on their racial characters should begin at once before depopulation begins on an alarming scale and miscegenation destroys the native purity of their blood.

The most important of these tribes are the Austric-speaking Mundas, Santals, Hos and the allied tribes and the Dravidian-speaking Oraons. It is proposed to carry on systematic investigations of all these groups from an anthropometric standpoint supplemented by a study of the skeletal

¹ H. H. Risley, *The People of India*, p. 48 (Calcutta, 1915).

materials that are available in the local collections. Being the most important group amongst them, the Mundas are selected first for the purpose of my present investigation.

I. THE ANTHROPOMETRIC MEASUREMENTS AND OBSERVATIONS

In the beginning of February 1933 I went to Ranchi for this purpose and consulted Rai Bahadur Sarat Chandra Roy, our distinguished authority on these tribes, and selected the villages in the Khunti area of the Ranchi district close to Jamri on the eastern side of the Tajna river for the investigation of the physical characters of the aboriginal Mundas. This is because the Mundas of that area have still preserved their primitive manners and customs in a state of comparative purity and most of them do not understand any other language except Mundari. The Christian Mundas and the *Khangars*, who seem to be allied to the original tribe, are not included in the present study; only the *Samsar*, or the true aboriginal Mundas, have been selected so as to avoid the possibility of the inclusion of any foreign element.

In the course of my investigations I was able to take anthropometric measurements of 250 different individuals. They belong to the following *Kilis* or totemistic septs. The figures within the brackets indicate the number of individuals measured belonging to each particular *Kili*: Tuti (153), Horo (26), Bing (8), Hāsā (7), Hāsā āḍā (12), Oḍe (3), Tāo (8), Chūtū Pūrthi (13), Kamal (4), Hans (6), Mundu (4), Sāngā (4) and Rundia (2).

Of these the Tuti forms the largest group (153 out of 250 individuals). The persons belonging to this *Kili* style themselves as Kompāt (Konkpat) Mundas.

They were all adult males and their ages varied from 25 to 50 years, and they were in a perfect state of health. Those persons in whom the teeth had fallen out have been carefully excluded, for in them the gradual absorption of the dental alveoli would lead to erroneous results.

The measurements were taken with the greatest care and precision and conform to the standard laid down in the International Agreement. The individuals measured belong to the following villages: Jamri, Barbanda, Murhi, Chamri, Dokar, Barkargi, Kanki, Gargaon, Taro, Jilinga, Deo, Patratoli, Posea, Senegutu, Sodag, Chikor, Karatoli, Kumkuma, Teram, Sarjema, Alaundi, Ondra, Bhandra.

The statistical constants of the measurements and indices on the living are given in Table VI.

THE PHYSICAL CHARACTERS

The Mundas have sturdy and well-formed limbs with well-developed musculature. The muscles of the shoulder are usually found to be very strong, due possibly to their habit of carrying loads on the Indian *bhāngi* poles.

TABLE I.—SKIN COLOUR

Scale number	Forehead	Breast	Upper arm
25	12	3	3
26	2	4	4
27	64	40	40
28	160	47	47
29	6	151	151
30	5	4	4
32	1	1	1

The skin colour was examined with Luschan's Hautfarbentafel on the forehead, chest, and inner side of the upper arm, and was found to vary from number 25 to 32. The commonest shades are 27, 28 and 29. In comparing the skin colour with the numbers on the scale it was noticed that in some cases the skin colour did not actually correspond to the scale number but only approached it. Great care was taken while matching the skin colour to the scale to bring the light reflection to the minimum. Table I gives the frequency of the results arrived at.

The hair is found to be black and corresponds in most cases to number 27 of the Haarfarbentafel of Prof. Dr. Eugen Fischer. In a few cases there is a slight brownish tint and in 5 individuals it approached number 6 of the above scale. It is possible that the change in colour may be due to the Munda habit of cleansing the head with a kind of earth, found in the neighbourhood, which is known popularly as Naḍka Hāsā.¹ The hair is usually wavy, in a few cases straight and not infrequently curly, but I have never met a single individual with woolly hair. They often keep the hair long and the Munda youths are fond of soaking their hair with *karanj* oil, after which it is tied in a knot on one side of the head with a small wooden comb stuck into it. In those cases where the hair is cut short a pigtail is often found on the head of a non-Christian Munda. The body hair is usually scanty, but occasionally quite thick. The moustache and beard are often shaved. And it is not unusual to find the

¹ Chemical analysis of the Naḍka Hāsā was kindly undertaken by Prof. N. C. Nag, and the following results were arrived at :

Moisture (loss at 100° C.)	= 3.56 per cent.
Loss on ignition	= 5.95
Al ₂ O ₃	= 15.69
FeO	= 0.25
Fe ₂ O ₃	= 10.65
MnO	= 0.03
CaO	= 0.72
MgO	= 0.59
K ₂ O, Na ₂ O (Alkali)	= 2.18
P ₂ O ₅	trace only
SiO ₂	= 59.46
TiO ₂	= 0.82

Total determined = 99.90

hair on the sternum shaved also. The eyebrows are usually medium, often they are scanty and sometimes they are found to be connected in the middle.

The colour of the iris is dark brown and corresponds in general to numbers 2 or 3 of the Augenfärbentafel of Prof. Dr. Rudolf Martin. The direction of the eye-slits is either horizontal or only slightly oblique, in which the external canthi are found to be on a slightly higher level than the inner ones. The true epicanthic or the 'Mongolian fold' was found in two cases only, while in seven individuals only traces of it were present.

The stature varies mainly from short to medium. The mean stature obtained by me is 1581.52 ± 2.04 mm. and the standard deviation 47.73 ± 1.44 . On analysing, the following results are obtained :

TABLE II.—STATURE

	Frequency	Percentage
Pigmy .	3	1.20 per cent.
Short .	123	49.20 „
Medium .	119	47.60 „
Tall .	5	2.00 „
Very tall .	Nil	Nil

Comparing these figures with those published by the late Sir Herbert Risley¹ on the Mundas of the Lohardaga area we find :

¹ H. H. Risley, 'The Tribes and Castes of Bengal,' *Anthropometric Data*, vol. i, pp. 385-398 (Calcutta, 1891).

TABLE III.—STATURE (IN MILLIMETERS)

	Measured by P. C. Basu (250 Persons)	Published by Risley (100 Persons)
Average . . .	1581.52	1589
Maximum . . .	1716	1718
Minimum . . .	1463	1446

Table III shows that there is only slight difference between the two series either in the mean or in the maximum and minimum statures.

The Mundas are mainly longheaded. Of the 250 individuals measured, 177 are Dolichocephalic, 72 Mesocephalic and only one Brachycephalic. The mean Cephalic index is

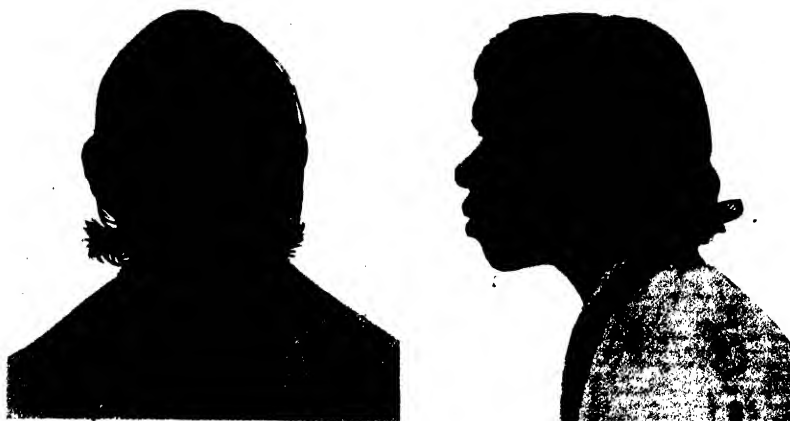


FIG. 119. Dolichocephalic chamærrhine adult Munda male.

74.34 ± 0.12 and the standard deviation is 2.75 ± 0.08 . Comparing my results with those given by Risley, I find a difference of only 0.16 in the average Cephalic index and 0.36 and 0.31 in the maximum and minimum values.

The mean Maximum head length of the men measured by me is 186.95 ± 0.24 mm. and the standard deviation 5.67 ± 0.17 , while the mean Maximum head breadth is 138.09 ± 0.18 mm., with the standard deviation 4.21 ± 0.13 . The average Cephalic length given by Risley is 185.9 and the Cephalic breadth 138.6.

The two series are, therefore, found to agree very closely.

The Auricular height of the head is taken to be the height of the vertex from the tragon of the ear. The mean Auricular height in the present series is 120.33 ± 0.27 mm., and the standard deviation 6.24 ± 0.19 . The mean Length-height index is 64.40 ± 0.15 and the standard deviation 3.43 ± 0.10 . The mean Breadth-height index in the present series is 86.69 ± 0.20 , and the standard deviation 4.70 ± 0.14 . On analysis we arrive at the following results :

TABLE IV.—BREADTH-HEIGHT INDEX OF HEAD

	Frequency	Percentage
Tapeinocephalic .	13	5.20
Metriocephalic .	78	31.20
Acrocephalic .	159	63.60

The nose is broad, with a mean Nasal index of 83.29 ± 0.30 , and the standard deviation 7.11 ± 0.21 . The average Nasal index obtained by Risley was 89.9. The difference between the two series is 6.61 and is quite significant. The mean Nasal length of the men measured by me is 48.41 ± 0.13 mm., with a standard deviation of 3.06 ± 0.09 , and the mean Nasal breadth is 40.18 ± 0.11 mm. with a standard deviation of 2.62 ± 0.08 . The average Nasal length and Nasal breadth obtained by Risley are 44.47 and 40.2 mm. respectively. The reason for the discrepancy between Risley's measurements and mine lies

therefore in the values of the Nasal length where a difference of 3.94 mm. is found. In his 'Anthropometric Instructions' in measuring the 'Nasal height' Risley advises¹ that 'the pointed end of the lower limb (of the nasometer) should be placed at the junction of the central cartilage with the upper lip, pressed inwards and upwards until it meets with steady resistance from the nasal spine.' The upper point is also easy to find in persons who have the root of the nose well defined. By feeling with the finger one can readily fix the point at which the bridge of the nose meets



FIG. 120. Mesocephalic chamærrhine adult Munda male.

the frontal region of the skull and forms a depression or valley, the deepest point of which determines the measurement.' In my measurements on the other hand, the upper point was always considered to be the anatomical point 'nasion' which was felt with the utmost care, and not the 'deepest point' advocated by Risley. Similarly with regard to the lower point pressure was never applied. The mean Nasal elevation index in the present series is 47.67 ± 0.21 , with a standard deviation of 4.89 ± 0.15 .

¹ H. H. Risley, 'Anthropometric Instructions,' p. 3. Appendix, *Jour. Asiatic Soc. Bengal*, vol. lxii, part iii (Calcutta, 1893).

By combining the head form with the nose form, we arrive at very interesting results :

TABLE V.

	Frequency	Percentage
Dolichocephalic leptorrhine .		0.80
Dolichocephalic mesorrhine .	103	41.20
Dolichocephalic chamærrhine .	69	27.60
Dolichocephalic hyperchamærrhine	3	1.20
Mesocephalic mesorrhine .	46	18.40
Mesocephalic chamærrhine .	24	9.60
Mesocephalic hyperchamærrhine	2	0.80
Brachycephalic chamærrhine .		0.40

The above table shows that the predominant element is Dolichocephalic mesorrhine; the next important groups are Dolichocephalic chamærrhine and Mesocephalic mesorrhine.

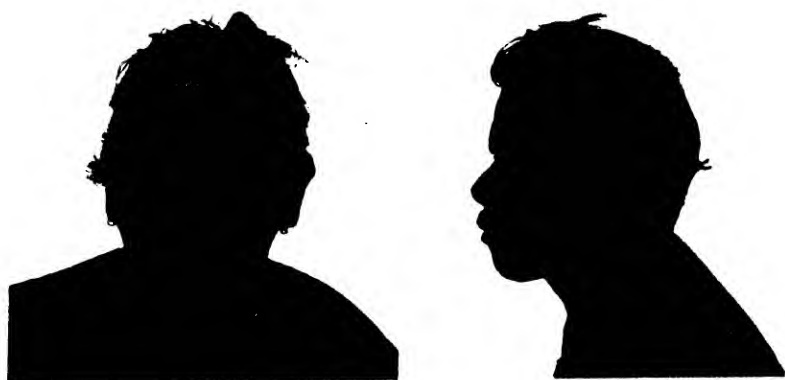


FIG. 121. Brachycephalic chamærrhine adult Munda male.

Neither Brachycephaly nor Leptorrhiny is present in any appreciable form.

In Fig. 119 are given the front and the profile views of a Dolichocephalic chamærrhine individual named Kanhu

from Alaundi, who has a Cephalic index of 72.77 and a Nasal index of 86.27 .

Similarly in Fig. 120 are given the front and the side views of Jado of Jamri, who has a Cephalic index of 76.60 and a Nasal index of 86.67 .

The only Brachycephalic individual in the present series is given in fig. 121. He is Turi Pahan of Jilinga. His Cephalic index is 81.36 and the Nasal index 85.42 .

The photographs given in figs. 119, 120 and 121 show the principal Chamærrhine types which form 39.6 per cent. of the whole series.

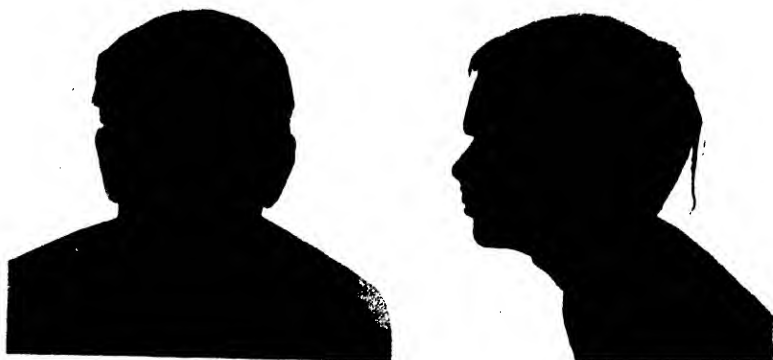


FIG. 122. Dolichocephalic mesorrhine adult Munda male.

The largest group in the present series, as already mentioned, is, however, Dolichocephalic mesorrhine (41.20 per cent.). An illustration of this type will be found in fig. 122, which gives the full face and the lateral profile views of Chankia, living in Posea near Jamri. His Cephalic index is 68.69 and the Nasal index 82.61 .

The Dolichocephalic leptorrhine element is represented by two individuals only. Like the Brachycephalic chamærrhine type its place in the racial constitution of the true Mundas is altogether negligible.

The Mesocephalic mesorrhine element is illustrated in figs. 123 and 124. Of these, fig. 123 gives the photographs of Rasai Munda of Bhandra, whose Cephalic index is 78.89

and the Nasal index 78.00. In fig. 124 are given the full face and the profile views of Mani Pahan of Bhandra. His Cephalic index is 76.72 and the Nasal index is 82.00. This Mesocephalic mesorrhine element forms 18.40 per cent. of the whole series.

In the Mundas measured by me, the forehead is either slightly retreating or arched. The supraorbital ridges are variable. They are usually moderately developed. But it is not uncommon to find individuals having a more or less vertical or bulging forehead, with the supraorbital ridges only

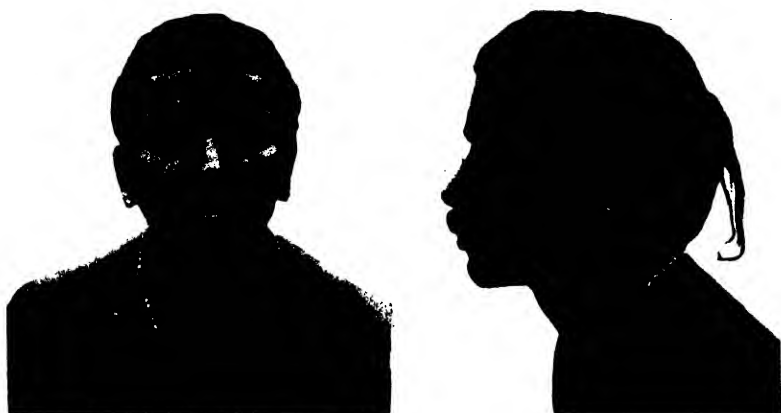


FIG. 123. Mesocephalic mesorrhine adult Munda male.

faintly marked. Similarly, persons with strongly developed supraorbital ridges and a low retreating or slightly arched forehead are also fairly often met with. The mean Least frontal breadth is 101.92 ± 0.16 mm., with a standard deviation of 3.75 ± 0.11 . The average Minimum frontal breadth given by Risley¹ is 101.5 mm. The difference between the two is therefore only 0.42 mm.

The root of the nose is, as a rule, depressed; occasionally the depression is not marked and very rarely it is absent. The nasal bridge is straight; sometimes it is found to be

¹ H. H. Risley, 'The Tribes and Castes of Bengal,' *Anthropometric Data*, vol. i, p. 398 (Calcutta, 1891).

concave or slightly convex or even sinuously curved. The nasal septum is generally horizontal, only rarely the nasal tip is found to be slightly turned up.

The mean Orbitonasal index is 113.20 ± 0.14 with a standard deviation of 3.40 ± 0.10 . The mean Orbitonasal breadth is found to be 95.46 ± 0.16 mm., with a standard deviation of 3.80 ± 0.11 , while the mean Orbitonasal arc is 108.04 ± 0.21 , with a standard deviation of 4.94 ± 0.15 .

The direction of the eye slits, as already mentioned, is

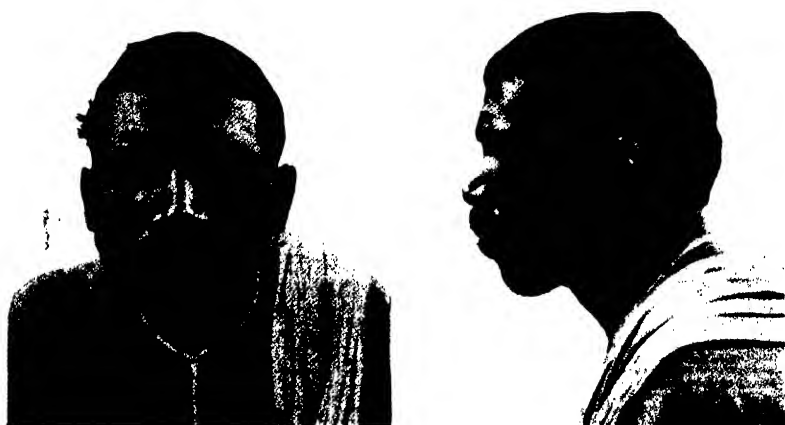


FIG. 124. Mesocephalic mesorrhine adult Munda male.

either horizontal or only slightly oblique. And the obliquity is due to the fact that the outer canthi are usually slightly on a higher level than the inner ones. The true epicanthic fold is present in two individuals only, and in seven cases there is a slight hint of it. The mean Interorbital breadth, that is, the distance between the two inner canthi, is 33.01 ± 0.10 mm., with a standard deviation of 2.39 ± 0.07 .

The face is generally pear-shaped. Sometimes the Maximum bizygomatic breadth exceeds the Maximum head breadth and the lateral prominence of the zygomatic bones gives the face a peculiar rhomboid outline. The mean

Maximum bizygomatic breadth is 131.70 ± 0.17 mm., with a standard deviation of 4.04 ± 0.12 . The average Maximum bizygomatic breadth given by Risley¹ is 130.7 mm. The difference between the two series is only 1.00 mm. The mean Bigonial breadth is 99.52 ± 0.20 , with a standard deviation of 4.65 ± 0.14 .

In the Total Facial Index we find the mean Total Facial Index is 84.90 ± 0.19 , with a standard deviation of 4.54 ± 0.14 , and of the 250 individuals 77 (30.80 per cent.) are Euryprosopic, 75 (30.00 per cent.) Mesoprosopic, 61 (24.40 per cent.) Leptoprosopic, 28 (11.20 per cent.) Hypereuryprosopic, and only 9 (3.60 per cent.) are Hyperleptoprosopic.

The teeth are good and well formed, but in some cases it is found that the crowns of the teeth are considerably eroded. Caries and pyorrhoëa alveolaris are not common. The jaws are well formed and powerful. Small and delicate jaws are met with only rarely.

To sum up, the Mundas have sturdy and well-formed limbs, with a well-developed musculature. The colour of the skin is chocolate brown approaching black. The hair is also black and usually wavy or curly, but not woolly or frizzly. The iris is dark brown. The eye slits are either horizontal or only slightly oblique, in which the outer canthi are slightly on a higher level than the inner ones. The true epicanthic or the Mongolian fold is almost absent—being found in two cases only. The stature is short. The head is undoubtedly long and narrow but not large. The forehead is usually slightly retreating and in some cases arched. The supraorbital ridges are generally well developed, but it is not uncommon to find persons having a bulging forehead with supraorbital ridges only faintly marked. Similarly individuals with well-developed supraorbital ridges are also often met with. The nose is broad and depressed at the root. The nasal bridge is, as a rule, straight. The face is generally pear-shaped. The teeth are good and well formed, but, probably due to the nature of their food, are worn down early.

¹ H. H. Risley, 'The Tribes and Castes of Bengal,' *Anthropometric Data*, vol. i, p. 398 (Calcutta, 1891).

TABLE VI.—STATISTICAL CONSTANTS OF THE MEASUREMENTS AND INDICES OF MUNDA MALES

Measurements and Indices	Number	Mean	Standard Deviation	Coefficient of Variation
1. Stature . .	250	1581.52 ± 2.04	47.73 ± 1.44	3.02 ± 0.09
2. Auricular height of head . .	250	120.33 ± 0.27	6.24 ± 0.19	5.18 ± 0.15
3. Maximum head length. .	250	186.95 ± 0.24	5.67 ± 0.17	3.03 ± 0.09
4. Maximum head breadth . .	250	138.09 ± 0.18	4.21 ± 0.13	3.05 ± 0.09
5. Least frontal breadth . .	250	101.92 ± 0.16	3.75 ± 0.11	3.68 ± 0.11
6. Maximum bizygomatic breadth . .	250	131.70 ± 0.17	4.04 ± 0.12	3.07 ± 0.09
7. Bigonial breadth	250	99.52 ± 0.20	4.65 ± 0.14	4.67 ± 0.14
8. Interorbital breadth . .	250	33.01 ± 0.10	2.39 ± 0.07	7.24 ± 0.22
9. Orbitonasal breadth . .	250	95.46 ± 0.16	3.80 ± 0.11	3.98 ± 0.12
10. Orbitonasal arc	250	108.04 ± 0.21	4.94 ± 0.15	4.57 ± 0.14
11. Nasal length . .	250	48.41 ± 0.13	3.06 ± 0.09	6.32 ± 0.19
12. Nasal breadth. .	250	40.18 ± 0.11	2.62 ± 0.08	6.52 ± 0.20
13. Nasal depth . .	250	19.10 ± 0.08	1.87 ± 0.05	9.79 ± 0.30
14. Upper facial length. . .	250	63.10 ± 0.18	4.17 ± 0.12	6.61 ± 0.20
15. Total facial length. . .	250	111.75 ± 0.23	5.48 ± 0.16	4.90 ± 0.15

TABLE VI.—STATISTICAL CONSTANTS OF THE MEASUREMENTS AND INDICES OF MUNDA MALES (*continued*)

Measurements and Indices	Number	Mean	Standard Deviation	Coefficient of Variation
16. Horizontal circumference of head . .	250	534.36 ± 0.51	12.01 ± 0.36	2.24 ± 0.07
17. Sagittal arc of head . .	250	343.64 ± 0.52	12.13 ± 0.37	3.53 ± 0.11
18. Transverse arc of head . .	250	319.60 ± 0.42	9.86 ± 0.30	3.08 ± 0.09
19. Length-breadth index of head	250	74.34 ± 0.12	2.75 ± 0.08	3.69 ± 0.11
20. Length - height index of head	250	64.40 ± 0.15	3.43 ± 0.10	5.32 ± 0.16
21. Breadth-height index of head	250	86.69 ± 0.20	4.70 ± 0.14	5.42 ± 0.16
22. Orbitonasal index . .	250	113.20 ± 0.14	3.40 ± 0.10	3.00 ± 0.09
23. Nasal index . .	250	83.29 ± 0.30	7.11 ± 0.21	8.53 ± 0.26
24. Nasal elevation index . .	250	47.67 ± 0.21	4.89 ± 0.15	10.26 ± 0.31
25. Upper facial index . .	250	47.96 ± 0.14	3.32 ± 0.10	6.92 ± 0.21
26. Total facial index . .	250	84.90 ± 0.19	4.54 ± 0.14	5.35 ± 0.16
27. Transverse cephalofacial index . .	250	94.86 ± 0.12	2.81 ± 0.08	2.96 ± 0.09
28. Vertical cephalofacial index . .	250	93.09 ± 0.27	6.32 ± 0.19	6.79 ± 0.20

II. THE MUNDA CRANIA

In the preceding section I have discussed the somatic characters of the Mundas based on the measurements of 250 adult males taken during my recent visit to their country. In the following pages an attempt will be made to compare them with investigations on Munda crania. Unfortunately reliably known aboriginal skulls are rare. But by the kind permission of Lt.-Col. R. B. Seymour Sewell, Director, Zoological Survey of India, and Dr. B. S. Guha, Anthropologist, Zoological Survey of India, I was fortunate enough to be able to examine the very important collection of Munda crania that is at present preserved in the Anthropological Laboratory of the Zoological Survey of India in the Indian Museum, Calcutta. Some of the skulls of this series were examined by Sir William Turner¹ and Mr. B. A. Gupte² some years ago. My measurements, however, conform to the technique advocated by the International Agreement supplemented by recent advances in Comparative Craniometry. Details as regards the locality and identity of the crania are taken from the Official Record. The numbers of the skulls mentioned in this paper refer to the official numbers in the Register of the Zoological Survey of India. There are altogether seven Munda skulls in the collection of the Indian Museum, of which five belonged to the male and two to the female sex. They were obtained by W. H. P. Driver and Lt.-Col. Dalton.

Of these the skull numbered $\frac{S. 25}{F. 12}$ is of a male individual from Jurobaree aged about 18 years. His living height as given in the Record was 5 ft. 4 ins. The skull 603 is of a Munda named Hochar, of the village Lodha, and aged about 45 years. The skull 606 is of an individual named Mangra Munda, of Ranchi (Old Town). The skull $\frac{S. 26}{F. 13}$ is that of

¹ W. Turner, 'Contributions to the Craniology of the Peoples of the Indian Empire,' Part iii, *Trans. Roy. Soc. Edin.*, vol. xl, pp. 59-130 (Edinburgh, 1905).

² B. A. Gupte, *Craniological Data from the Indian Museum*, p. 47 (Calcutta, 1909).

Dhiroo Munda, male, from Kakadeh, and aged 32 years. His living height as given in the Register was 5 ft. 5 ins. The skull 607 is of Lelle Munda, from Konka (Ranchi), and belonged to a woman. The skull marked 611 is of Gonda from Lalpur. It is greatly damaged, only the cranial vault is left; it belonged to a female. The skull 612 belonged to an adult Munda male named Debia, of Lalpur. All the skulls mentioned above are mostly in a good state of preservation and were identified before they were removed from the dead body by persons who were acquainted with the deceased.

THE CRANIAL CAPACITY

The cranial capacity of the skulls in the present series has been calculated according to the Formulæ Nos. 10 and 11 of Alice Lee and Karl Pearson.¹ In the Munda males the capacity varies from 1259 c.c. to 1410 c.c. The two female Munda crania show cranial capacities of 1100 c.c. and 1231 c.c. The average cranial capacity of the Munda males is 1342.60 c.c. But if we exclude from the series the skull numbered $\frac{S. 25}{F. 12}$, which belonged to an individual aged 18 years, the average cranial capacity rises to 1363.5 c.c.

THE POSITION OF THE EXTERNAL AUDITORY MEATUS

The position of the External Auditory Meatus in the skulls unfortunately has not received the attention it deserves. Following the method advocated by Sewell and Guha² the position of the auditory meatus was calculated in these skulls.

The average Meatal position index in the adult Munda ♂ was found to be 53.69. The average Meatal position index

¹ Alice Lee and Karl Pearson, 'A First Study of the Correlation of the Human Skulls,' *Phil. Trans. Roy. Soc. London*, Series A, vol. 196, p. 241 (London, 1901).

² R. B. S. Sewell and B. S. Guha, *Report on the Human Remains excavated at Mohenjodaro and the Indus Valley Civilisation in Mohenjodaro*, edited by Sir John Marshall, vol. ii, chap. xxx, p. 607 (London, 1931).

obtained by Sewell and Guha¹ in three adult skulls from Mohenjodaro (Proto-Australoid type) was 48·1, in 5 Veddahs 51·43, in 20 Tasmanians 51·49, in 20 Australians 53·01, in 3 Aditannalur skulls 54·60, and 55·5 was obtained by the present author in 30 male Burmese crania from an old burial ground in Prome.²

GENERAL CHARACTERISTICS OF THE SKULLS

In the Munda skulls the supraorbital ridges are usually well developed in the males, and sometimes they are so prominent that they strongly resemble the Australian, Melanesian and the Tasmanian skulls so as to form a supra-orbital torus as in Munda 606 and 603 (see figs. 125 and 126). For detailed explanation of these figures the reader is referred to the beginning of page 232. It is interesting to note that similar prominent supraorbital ridges were noticed in the human relics recovered by the Naga Hills (Burma) Expedition for the Abolition of Human Sacrifice during 1926-27.³

The forehead is usually slightly receding, sometimes markedly so. The average Least frontal breadth for the adult Munda ♂ is 90·75 mm. and the average Greatest frontal breadth is 109·25 mm. The relation of the Least frontal breadth with the Maximum cranial breadth as judged from the Transverse frontoparietal index shows that only one is metriometopic, while the rest are eurymetopic, the mean for the adult Munda ♂ being 69·77.

The sutures of the cranial vault as a rule are distinct and small wormian bones are found, especially along the parieto-occipital suture. In Munda 606, a triangular piece of bone (Os Inca or Peruvian bone) is present at lambda. It measures 41 mm. × 34 mm.

In the same skull a small epipteric bone is present on either side, and in the skull numbered 607 a small wormian bone is found at Pars asterica on the right side. In Munda

¹ R. B. S. Sewell and B. S. Guha, *loc. cit.*, p. 639.

² P. C. Basu, 'A Comparative Study of Burmese Crania,' *Trans. Bose Research Inst.*, vol. vii, p. 274 (Calcutta, 1931-32).

³ B. S. Guha and P. C. Basu, 'Report on the Human Relics recovered by the Naga Hills (Burma) Expedition for the Abolition of Human Sacrifice during 1926-27,' *Anthropological Bulletin No. 1, Zoological Survey of India*, p. 15 (Calcutta, July 1931).

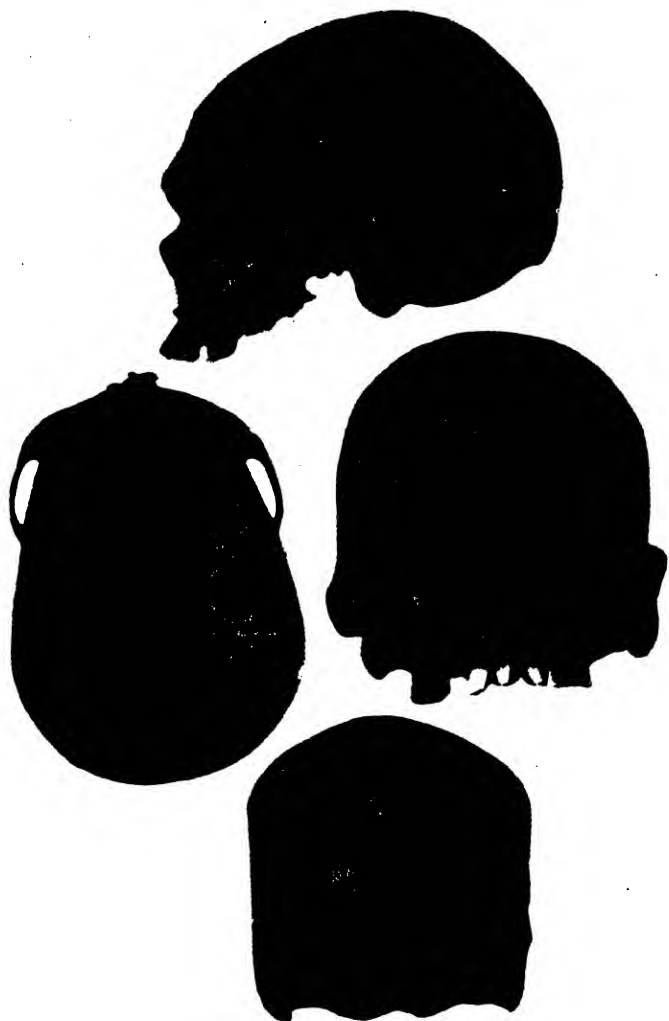


FIG. 125. Dolichocranial chamærrhine.

♂ $\times \frac{5}{18}$ (Skull No. 606).

$\frac{S. 25}{F. 12}$ the squama on the left temporal bone articulates with the frontal, thus cutting off the anterior inferior angle of the parietal from joining the great wing of the sphenoid.

The nature of the sutures on the cranial vault and the

degree of their complications are given in Table VII, arranged according to the scheme of Oppenheim.¹

TABLE VII.—SUTURE CHARACTERISTICS OF THE MUNDA CRANIA

Skull No.	603	606	607	612	S. 25 F. 12	S. 26 F. 13	611
<i>Coronal suture :</i>							
Pars bregmatica	II ₂	II ₃	II ₂	II ₃	II ₂	III ₂	II ₂
Pars complicata	IV ₉	IV ₄	II ₄	IV ₇	I ₂	IV ₇	III ₄
Pars temporalis	I ₃	I ₁	I ₁	—	I ₁	I ₂	III ₂
<i>Sagittal suture :</i>							
Pars bregmatica	II ₃	II ₃	II ₃	III ₅	II ₃	—	II ₂
Pars verticis	II ₆	II ₅	II ₆	III ₅	I ₉	—	II ₅
Pars obelica	I ₂	II ₃	I ₃	—	II ₃	—	I ₃
Pars postica	II ₃	II ₆	IV ₇	II ₅	II ₇	—	III ₈
<i>Lambdoid suture :</i>							
Pars lambdoidea	II ₃	II ₁₀	IV ₄	(R) II ₈ (L) III ₅	IV ₈	IV ₉	II ₃
Pars media	IV ₉	III ₁₀	IV ₈	(R) II ₉ (L) II ₈	III ₉	II ₆	III ₈
Pars asterica	II ₃	I ₁	II ₃	WB	I ₃	I ₂	II ₃

EXPLANATION.

Confer Martin's *Lehrbuch der Anthropologie*, Bd. II, S. 732-3 (Jena, 1928). The Roman numerals refer to the main or principal group of suture characters of Oppenheim.

The numerals a little below the group number mean subdivisions of that group.

R = Right ; L = Left ; WB. = Wormian bone.

The glenoid fossæ are usually deep. In Munda 612 is found a coronal depression about 1½ ins. wide just behind the coronal suture.

¹ R. Martin, *Lehrbuch der Anthropologie*, 2 Auf., Bd. II, S. 732-3 (Jena, 1928).

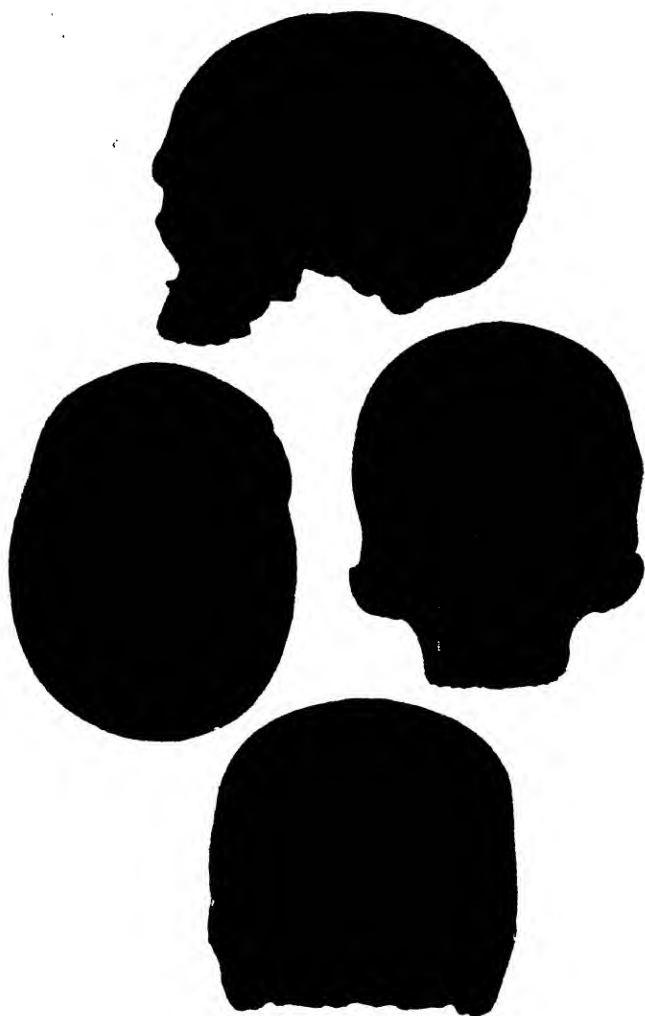


FIG. 126. Dolichocranial chamærrhine.
 $\sigma \times \frac{6}{18}$ (Skull No. 603).

The Munda skulls are all dolichocranial. The average Maximum cranial length of the adult Munda males is 179 mm. and the Maximum cranial breadth is 130 mm. The average Cranial index of the adult Munda σ is 72.64. In

figs. 125 and 126 are given the photographs of two Munda male skulls numbered 606 and 603. In these the upper illustration is the side view, the middle left illustration is the view from the top, the middle right is the front view, while the lowest photograph gives the view of the skull from behind. It should be noticed that in the front view of the Munda skull No. 606 the mastoid processes project on either side of the maxilla.

Coming to the Length-height index we find the skulls are mainly Orthocranial. The average Length-height index of the adult Munda ♂ is 72.63 and the average Basilobregmatic height is 130 mm.

In the Breadth-height index of the cranium we find the skulls to be mainly Acrocranial, with only one Metriocranial skull among the adult Munda ♂ crania. The average Breadth-height index for the adult Munda ♂ skull is 100.03.

The nose is broad. All the Munda crania belong to the chamærrhine group; the average Nasal index for the adult Munda ♂ crania is 52.65.

The orbits are variable on the two sides. The average Orbital index for adult Munda ♂ is 75.82 on the right side and 81.33 on the left. The Orbito-nasal index is also found to be a variable one. In the adult Munda ♂ series we find 1 Prosopic, 2 Platyopic and 1 Mesopic. The average Orbito-nasal index in it is 108.80.

The Superior facial index shows the Munda ♂ (adult) to be euryene and mesene, the average Superior facial index being 49.05. (The average Nasion prosthion line in the adult Munda ♂ is 62 mm. and the Maximum bizygomatic breadth is 126.5 mm.)

The Facial profile angle shows a range of variation from 80° to 90°, and we find that both the meso- and the orthognathous varieties are present. The average Facial profile angle in the adult Munda ♂ is 83°.

The Palate is well formed. In the Munda ♂ (adult) we find 1 to be brachystaphyline and 2 leptostaphyline. The Maxilloalveolar index shows that the alveolar process is considerably broad. All the adult Munda male skulls are

brachyuranic. The average Maxilloalveolar index in the Munda males (adult) is 123.21.

The teeth are well formed, but in Munda 607 the three upper molars and one incisor are affected with caries and in the lower jaw the teeth are full of tartar. In Munda 612 the upper teeth are eroded to a considerable degree. The erosion in the skull numbered $\frac{S. 26}{F. 13}$ is moderate.

The measurements and indices of the Munda crania are given in Tables VIII and IX respectively. The linear measurements are all given in millimeters.

TABLE VIII.—MEASUREMENTS OF MUNDA CRANIA

Number of the skull	603	606	607	612	S. 25 F. 12	S. 26 F. 13	611
Sex	♂	♂	♀	♂	♂	♂	♀
1. Maximum cranial length	178	183	165	179	176	176	169
2. Maximum cranial breadth	133	130	112	129	123	128	123
3. Nasioninion length	167	169	148	164	164	168	162
4. Basilobregmatic height	130	133	130	129	131	128	128
5. Least frontal breadth	96	87	88	91	86	89	—
6. Greatest frontal breadth	116	108	99	108	106	105	107
7. Bimastoid breadth	101	102	94	95	90	98	89
8. Bizygomatic breadth	129	128	116	128	123	121	—
9. Nasion basion line	100	94	98	94	90	100	94
10. Prosthion basion line	92	91	94	94	90	93	—
11. Nasion gnathion line	113	—	99	104	—	107	—
12. Nasion prosthion line	65	62	56	58	60	63	—
13. Nasal length	47	49	42	47	45	47	—
14. Nasal breadth	25	25	25	25	23	25	—
15. Interorbital breadth	17	21	19	18	17	21	17
16. Orbital breadth :							
Right	39	38	35	37	39	38	—
Left	39	37	35	35	40	39	—
17. Orbital height :							
Right	31	27	29	31	34	30	—
Left	32	28	30	30	34	32	—

THE RACIAL AFFINITIES OF THE MUNDAS

TABLE VIII.—MEASUREMENTS OF MUNDA

Number of the skull	603	606	607	612			
Sex	♂	♂	♀	♂			
18. Maxilloalveolar breadth	63	67	62	62	62	64	—
19. Maxilloalveolar length .	49	54	49	50	47	53	—
20. Palatal length . . .	45	58	49	—	40	55	—
21. Palatal breadth . . .	42	45	37	39	40	43	—
22. Occipital foramen :							
Length	33	35	31	34	35	35	33
Breadth	26	29	27	27	29	31	30
23. Sagittal cranial arc .	362	380	335	365	367	355	345
24. Transverse cranial arc .	300	280	268	287	275	275	277
25. Horizontal circumference	503	515	455	497	488	491	—
26. Bicondylar breadth (mandible)	110	—	—	112	—	114	—
27. Bigonial breadth . . .	90	88	92	90	—	89	—
28. (i) Length (height) of ramus	66	—	54	66	—	58	—
(ii) Breadth of ramus :							
(a) Minimum . . .	35	35	30	30	—	28	—
(b) Maximum . . .	46	43	39	40	—	41	—
29. Symphysial height . . .	34	31	28	—	—	28	—
30. Mandibular length . . .	61	72	62	65	—	61	—
31. Mandibular angle . . .	115°	116°	120°	120°	—	131°	—
32. Biauricular breadth . .	118	116	104	115	112	101	110

PROVASH CHANDRA BASU

TABLE VIII.—MEASUREMENTS OF MUNDA CRANIA (*continued*)

Number of the skull	603	606	607	612	S. 25 F. 12	S. 26 F. 13	611
Sex	♂	♂	♀	♂	♂	♂	♀
33. Outer biorbital breadth	102	104	98	101	99	104	—
34. Inner biorbital breadth.	96	98	99	96	92	97	—
35. Greatest occipital breadth	105	99	90	114	112	100	93
36. Frontal arc . . .	125	125	113	120	125	110	116
37. Parietal arc . . .	123	} 257*	{ 120	135	131	125	131
38. Occipital arc . . .	114		{ 102	110	111	120	98
39. Frontal chord . . .	110	111	99	106	108	96	101
40. Parietal chord . . .	109	—	107	121	117	114	113
41. Occipital chord . . .	91	—	87	92	90	98*	86
42. Length of first Premolar to third Molar . . .	—	50	40	—	—	—	—
43. Length of first Molar (Upper) :							
Right { Anteroposterior	10	10	9	8	10	10	—
Right { Transverse . . .	9	11	11	10	9	12	—
Left { Anteroposterior	10	10	9	8	9	10	—
Left { Transverse . . .	9	11	11	10	9	11	—
44. Length of second Molar (Upper) :							
Right { Anteroposterior	9	—	9	7	9	9	—
Right { Transverse . . .	9	—	11	10	11	11	—
Left { Anteroposterior	9	9	9	—	9	8	—
Left { Transverse . . .	10	12	11	—	10	12	—

* Wormian bone present.

TABLE VIII.—MEASUREMENTS OF MUNDA CRANIA (*continued*)

Number of the skull	603	606	607	612	S. 25 F. 12	S. 26 F. 13	611
Sex	♂	♂	♀	♂	♂	♂	♀
45. Biorbitonasal arc . . .	109	105	100	102	100	105	—
46. Glabella nasion length . . .	7	9	9	9½	7	12	13
47. Nasion lambda line . . .	172	176	158	172	171	170	161
48. Calvarial height . . .	106	112	100	103	103	103	104
49. Lambda calvarial height . . .	70	—	66	74	76	64	72
50. Bregma position line . . .	98	99	89	94	93	87	94
51. Frontal perpendicular . . .	25	23	24	25	26	22	25
52. Parietal perpendicular . . .	23	—	22	26	23	26	27
53. Occipital perpendicular . . .	27	—	23	24	27	29	22
54. Frontal inclination angle . . .	61°	66°	65°	64°	65°	65°	65°
55. Occipital inclination angle . . .	82°	—	87°	88°	84°	—	86°
56. Facial profile angle . . .	90°	80°	84°	81°	78°	81°	—
57. Calvarial base angle . . .	12°	15°	10°	12°	15°	10°	—
58. Frontal curvature angle . . .	132°	135°	130°	129°	130°	132°	129°
59. Parietal curvature angle . . .	134°	—	135°	135°	140°	132°	132°
60. Occipital curvature angle . . .	116°	—	125°	123°	120°	120°	123°
61. Occipital flexional angle . . .	116°	—	125°	124°	120°	120°	121°
62. Superior facial length angle	37°	38°	32°	35°	36°	35°	—
63. Nasion to foot of bregma perpendicular . . .	52	44	41	45	50	41	43
64. Cranial capacity in c.c. (calculated) . . .	1405	1410	1100	1379	1252	1260	1231

TABLE IX.—INDICES OF MUNDA CRANIA

Number of the skull	603	606	607	612	S. 25 F. 12	S. 26 F. 13	611
Sex	♂	♂	♀	♂	♂	♂	♀
<i>Indices of the cranium:</i>							
1. Length-breadth index	74·72	71·04	67·88	72·07	69·89	72·73	72·78
2. Length-height index	73·03	72·68	78·79	72·07	74·43	72·73	75·74
3. Breadth-height index	97·74	102·38	116·07	100·00	106·50	100·00	104·07
4. Calvarial height index	63·47	66·27	67·57	62·80	62·80	61·31	64·20
5. Bregma position index	31·14	26·03	27·70	27·44	30·49	24·40	26·54
6. Sagittal cranial curvature index	43·37	44·47	44·17	44·93	44·68	47·32	46·96
7. Transverse cranial curvature index	39·33	41·43	38·80	40·07	40·72	36·73	39·71
8. Transverse fronto-parietal index	72·18	66·92	78·57	70·54	69·92	69·53	—
9. <i>Indices showing the relations of the various sagittal arcs:</i>							
(a) Fronto parietal index	98·40	96·00	106·19	112·50	104·80	113·63	112·93
(b) Fronto occipital index	91·20	—	90·27	91·67	88·80	109·09	84·48
(c) Parieto occipital index	92·68	—	85·00	81·48	84·73	96·00	74·81
(d) Fronto sagittal arc index	34·53	32·63	33·73	32·87	34·06	30·98	33·62
(e) Parieto sagittal arc index	33·99	—	35·82	36·99	35·70	35·21	37·97
(f) Occipito sagittal arc index	31·49	—	30·44	30·13	30·24	33·80	28·40

TABLE IX.—INDICES OF MUNDA CRANIA (*continued*)

Number of the skull	603	606	607	612	$\frac{S. 25}{F. 12}$	$\frac{S. 26}{F. 13}$	611
Sex	♂	♂	♀	♂	♂	♂	♀
10. <i>Indices showing the amount of curvature (bulging) of each of the three contour bones of the cranium :</i>							
(a) Frontal curvature index	88.00	88.80	74.44	88.33	86.40	87.27	87.07
(b) Parietal curvature index	88.62	—	89.17	89.63	89.31	91.20	86.26
(c) Occipital curvature index	79.82	—	85.29	83.64	81.08	81.67	87.96
<i>Indices of the face :</i>							
11. Total facial index	87.68	—	85.34	81.25	—	88.43	—
12. Superior facial index	50.39	48.44	48.27	45.31	48.78	52.06	—
13. Zygomatico-frontal index	74.42	67.97	75.86	71.09	69.92	73.55	—
14. Zygomatico-mandibular index	69.77	68.55	79.31	70.31	—	73.55	—
15. Interorbital index	16.67	20.19	19.39	17.82	17.17	20.19	—
16. Orbital index :							
Right	79.49	71.05	82.85	83.78	87.18	78.95	—
Left	82.05	75.50	85.71	85.71	85.00	82.05	—
17. Nasal index	53.19	51.02	59.52	53.19	51.11	53.19	—
18. Maxilloalveolar index	134.04	124.07	126.53	124.00	131.91	120.75	—
19. Palatal index	93.33	77.59	75.51	—	100.00	78.18	—

TABLE IX.—INDICES OF MUNDA CRANIA (*continued*)

Number of the skull	603	606	607	612	S. 25 F. 12	S. 26 F. 13	611
Sex	♂	♂	♀	♂	♂	♂	♀
20. Mandibular index . . .	55.45	—	—	58.04	—	53.51	—
21. Ramus index . . .	69.70	—	70.22	60.61	—	70.69	—
22. Dental index . . .	—	53.19	40.81	—	—	—	—
<i>Indices showing relations between cranium and face :</i>							
23. Longitudinal craniofacial index . . .	35.61	33.88	34.57	32.40	51.14	35.79	—
24. Transverse craniofacial index . . .	96.99	98.46	103.57	99.22	100.00	94.53	—
25. Vertical craniofacial index . . .	50.00	46.61	43.07	44.96	45.80	49.22	—
<i>Some additional indices :</i>							
26. Lambda calvarial height index . . .	40.70	—	41.51	43.27	44.44	38.09	43.47
27. Frontal perpendicular index . . .	22.72	20.72	24.24	28.58	24.07	22.92	24.75
28. Parietal perpendicular index . . .	21.10	—	20.56	21.49	19.66	22.81	27.89
29. Occipital perpendicular index . . .	29.67	—	26.44	26.09	30.00	29.59	27.91
30. Meatal position index . . .	54.22	55.09	53.70	51.83	48.19	53.61	—
31. Orbitonasal index . . .	113.54	107.14	101.01	106.25	108.26	108.25	—

THE FACIAL PROJECTIONS

The study of Facial Projections in the determination of the racial differences is of considerable importance. Following Sir Arthur Keith ¹ an attempt has been made to determine the degree of the forward growth in the component

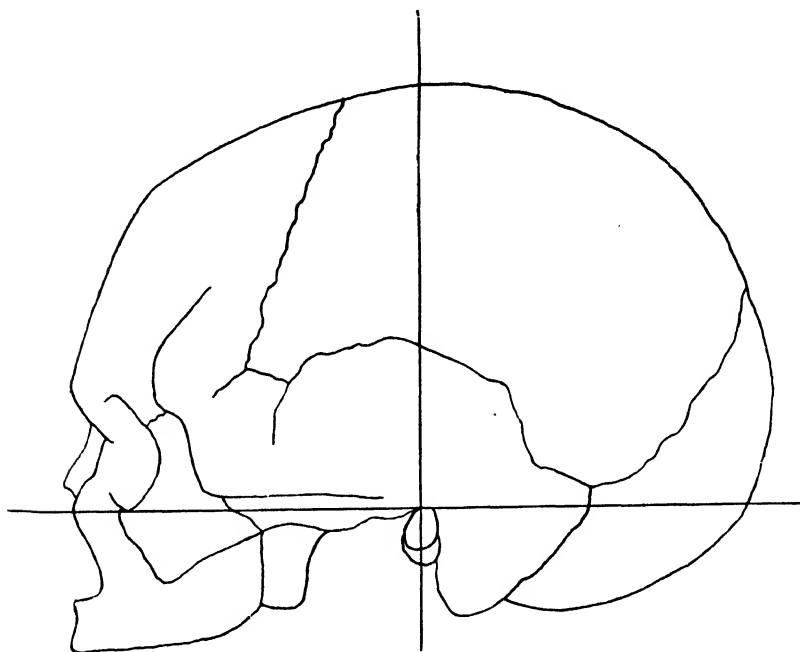


FIG. 127. Composite profile view of adult Munda ♂ crania $\times \frac{1}{2}$.

elements of the face in the adult Munda males by projections drawn in an anteroposterior plane. The measurements taken according to this method are given in Tables X and XI.

The glabellar projection from the midmeatal plane for adult Munda males is 90·25 mm., and the average projection of the nasion in front of the midmeatal plane is 84·75 mm.

¹ Sir Arthur Keith, 'Reports on the Human Remains' in *Ur Excavations*, vol. i (*Al-'U-Baid*), pp. 231-239, by H. R. Hall and C. L. Woolley (Oxford, 1927). See also *idem*, 'Human Skulls from Ancient Tarim Basin,' *Journ. Roy. Anthrop. Inst.*, vol. lix, pp. 168-171 (London, 1929).

The difference between the projection of the glabella and the projection of the nasion gives us the depth of the subglabellar notch at the root of the nose, and is found to be 5.50 mm. for adult Munda males. In Naga Group II recently studied¹ it was found to be 6.5 mm.; in the Mohenjodaro skulls of the Proto-Australoid type examined by Sewell and Guha² it was as high as 8.5 mm., while in the Burmese crania studied by me it was found to be 2.7 mm. for ♂ and 3.0 mm. for ♀.³ In the Tasmanian and in the Melanesian crania it is 7.0 and 6.7 respectively.⁴

The projection of the nasion in advance of the lateral orbital point is found to be 19.00 mm. for the adult Munda males. This projection was found to be 16.4 mm. for ♂ and 12.8 mm. for ♀ in the Burmese crania mentioned. In Naga II it is 14.8 mm., in Melanesian skulls 19.8 mm. and in the Tasmanian crania 18.5 mm.⁵

The difference between the projection of the lateral orbital point and the projection of the tip of the nose from the midmeatal plane gives us the amount of the forward projection of the tip of the nose from the lateral orbital point. It is found in the adult Munda ♂ to be 26.75 mm.

The distance between the most advanced part of the dorsum of the nose and the inferior orbital margin where it is crossed by the malomaxillary suture gives us another means of determining the forward projection of the nose. This difference is found in the adult Munda ♂ to be 16.25 mm.

The projection of the lower malomaxillary point in front of the midmeatal plane is 66.50 mm., while the projection of the least advanced part of the lateral margin of the pyriform aperture in front of the midmeatal plane is 81.75 mm. for the adult Munda ♂. The difference between the two is 15.25 mm.

The difference between the projection of the nasion and the projection of the upper alveolar point from the midmeatal plane gives us a very useful method for determining

¹ B. S. Guha and P. C. Basu, *loc. cit.*, p. 15.

² R. B. S. Sewell and B. S. Guha, *loc. cit.*, chap. xxx, p. 648.

³ P. C. Basu, *loc. cit.*, p. 310.

⁴ B. S. Guha and P. C. Basu, *loc. cit.*, pp. 65-66.

⁵ *Ibid.*

the nature of the upper alveolar prognathism. The average of this distance is found to be 6.00 mm. in the adult Munda males.

The three composite normæ (lateralis, frontalis and verticalis) of the adult Munda male crania are drawn in

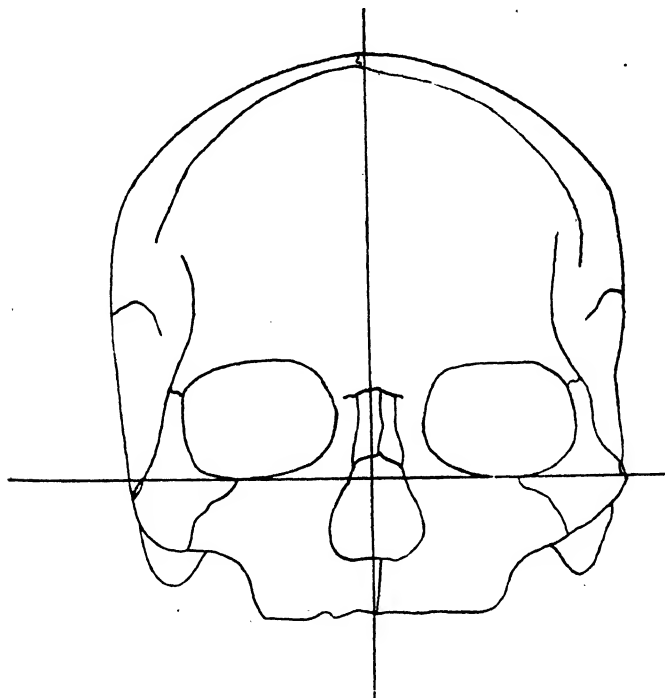


FIG. 128. Composite frontal view of adult Munda ♂ crania $\times \frac{1}{2}$.

accordance with the method recently elaborated by Prof. Frederic Wood-Jones¹ and are given in figs. 127-129.

From the accounts given it will appear that in the Munda skulls the supraorbital ridges and glabella are strongly marked, forming a supraorbital torus. The depth of the subglabellar notch is also great, giving the lower forehead a distinctly

¹ Frederic Wood-Jones, 'The Tasmanian Skull,' *Journ. Anatomy*, vol. lxii, pp. 224-230 (Cambridge, 1928-29).

Australoid appearance. The external auditory meatus is placed rather posteriorly, so that the preauricular development of the skull is somewhat greater than the postauricular. In this manner the Mundas, while agreeing with the Veddahs and the Australians in general, differ very significantly from

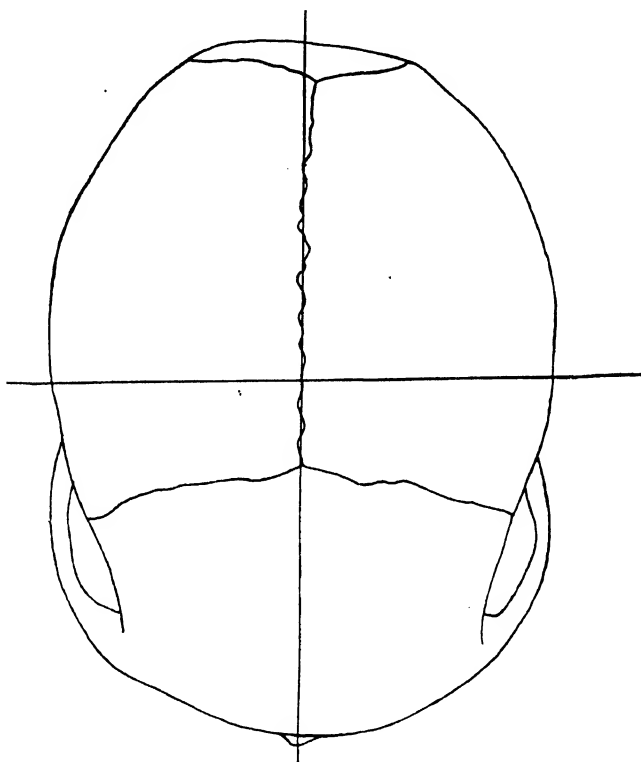


FIG. 129. Composite vertical view of adult Munda ♂ crania $\times \frac{1}{2}$.

the Proto-Australoid type of skulls found in the ancient civilisations of the Indus Valley. The skulls are all dolichocranial but are not very large. The vault of the cranium is not very high. The nose is broad. The palate is well formed. The alveolar process is considerably broad. The teeth are good and strong.

TABLE X.—MUNDA CRANIA (MALES)
Facial Measurements in an Anteroposterior Plane

Skull No.	A	B	C	D	E	F	G	H	I	J
603	93.0	93.0	66.0	88.0	89.0	80.0	87.0	88.0	68.0	18.0
606	93.0	99.0	70.0	86.0	96.0	87.0	95.0	97.0	69.0	21.0
612	84.0	87.0	64.0	80.0	85.0	80.0	86.0	89.0	65.0	13.0
S. 26 F. 13	91.0	91.0	63.0	85.0	89.0	80.0	85.0	89.0	64.0	13.0
Average	90.25	92.50	65.75	84.75	89.75	81.75	88.25	90.75	66.50	16.25

EXPLANATION

- A. Projection of the glabella in front of the midmeatal plane.
- B. Extent to which the tip of the nose lies in front of the midmeatal plane.
- C. Projection of the lateral orbital margin in front of the midmeatal plane.
- D. Projection of the nasion in front of the midmeatal plane.
- E. Projection of the most advanced point in the ascending nasal process of the maxilla in front of the midmeatal plane.
- F. Projection of the least advanced part of the lateral margin of the pyriform aperture in front of the midmeatal plane.
- G. Projection of the least advanced point just below the nasal spine in front of the midmeatal plane.
- H. Projection of the upper alveolar point in front of the midmeatal plane.
- I. Projection of the lower malomaxillary point in front of the midmeatal plane.
- J. Height of the most advanced part on the dorsum of the nose from the inferior orbital margin where it is crossed by the malomaxillary suture.

TABLE XI.—MUNDA CRANIA (MALES)
Projection of the Cheek Bones

Skull No.	A	B	C	D	E	F
603	75.0	69.0	79.0	69.0	40.0	17.0
606	77.0	73.0	79.0	70.0	42.0	23.0
612	73.0	68.0	76.0	66.0	42.0	20.0
S. 26 F. 13	77.0	67.0	78.0	64.0	41.0	21.0
Average	75.5	69.25	78.00	67.25	41.25	20.25

EXPLANATION

- A. The radial distance of the anterior end of the frontomalar suture from the transmeatal axis.
- B. The radial distance of the middle point on the lateral margin of the orbit from the transmeatal axis.
- C. The radial distance of the point on the lower margin of the orbit crossed by the malomaxillary suture from the transmeatal axis.
- D. The radial distance of the malomaxillary point from the transmeatal axis.
- E. The distance of the lower malomaxillary point from the anterior frontomalar point.
- F. The distance of the lower malomaxillary point to the nearest point on the lower margin of the orbit.

CONCLUSION

From the foregoing accounts we find that the results obtained from the study of the Munda crania are in the main in complete accord with those derived from the measurements of the 250 living Mundas from the interior of the Ranchi district. In both, the forehead is usually low and slightly retreating. The supraorbital ridges and glabella are prominent, forming a supraorbital torus in some cases. The root of the nose is depressed and broad. The skulls are all broad nosed, while in the living the average Nasal index is 83.29 , showing a tendency towards chamærrhiny. The head is predominantly long but not very large. All the skulls examined are dolichocranial, but in the living, dolichocephaly is represented by 70.80 per cent., and the rest though technically falling within mesocephaly have the characteristic occipital bulging shown by longheaded races without any flattening of the back of the head. The external auditory meatus is placed rather posteriorly in the Munda crania, so that the preauricular development of these skulls is greater than the postauricular. In this respect we find that the Munda crania, while resembling the Veddahs and the Australians in general, differ very significantly from the Proto-Australoid type of crania found in the prehistoric civilisations of the Indus Valley.

I take this opportunity of expressing my gratitude to Sir J. C. Bose for kind encouragement and active interest.

I am grateful to Lt.-Col. R. B. S. Sewell, the Director, Zoological Survey of India, for permission to work on and publish the measurements and exact reproductions of some of the Munda crania that are in the collection of the Anthropological Laboratory of the Zoological Survey of India, Indian Museum, Calcutta. I am greatly indebted to Dr. B. S. Guha, Anthropologist to the Zoological Survey of India, for special directions and advice in carrying out these investigations. My thanks are due to Prof. N. C. Nag, who has helped me in various ways, and to Mr. Taraprasanna Ghose of Ranchi for making arrangements for my field investigations among the primitive Mundas of Jamri and its neighbourhood.

XIII.—INTERPRETATION OF THE ABSORPTION SPECTRUM OF SILVER HALIDES

BY

ARUN KUMAR DATTA, D.Sc.

THE absorption spectra of silver halides have formed the subject of interesting controversy in recent years.¹ The

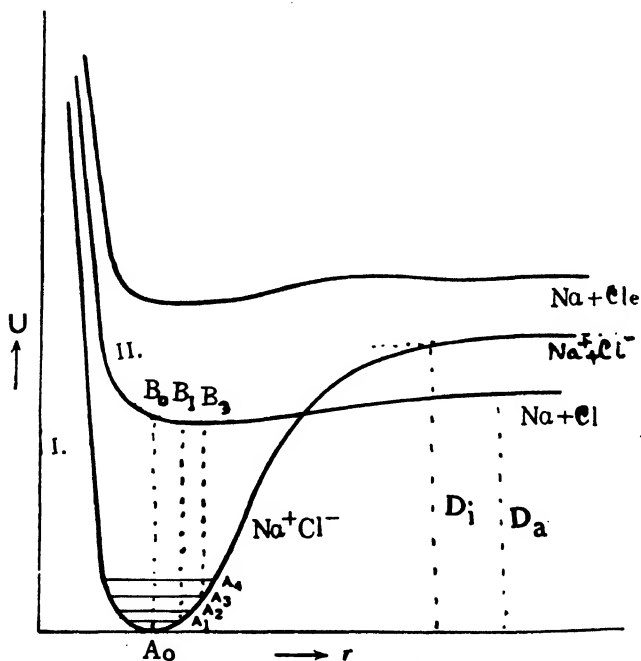


FIG. 130. Potential energy curves of NaCl in different states.

chemical properties of silver, as is well known, are identical with those of the alkalis, hence it is expected that the absorption of light by the vapour of the silver halides will

be similar to that of the alkali halides. Franck and his co-workers² have shown that the molecules of alkali halides, such as NaCl, show a continuous absorption beginning from a long wavelength limit. They explain the phenomenon in the following way: Crystals of NaCl, etc., form a cubic lattice composed of Na^+ and Cl^- ions, arranged regularly. When the crystal is vaporised we can suppose that the molecule consists of a combination of Na^+ and Cl^- ions, held together by electrostatic forces (polar compounds). When light falls on the NaCl molecule, it acts on the electron of Cl^- , which passes over to Na^+ , giving rise to neutral Na and neutral Cl. They thus ascribe the absorption spectrum to a photochemical process leading to decomposition of NaCl into atomic Na and atomic Cl, the light energy $h\nu_0$ being equivalent to R , the atomic heat of formation, where ν_0 is the frequency of the long wave-length limit of absorption.

The above explanation is illustrated in fig. 130, adapted from a recent paper of Franck.³ The abscissæ represent the distance between the two constituents, U their mutual potential energy. In the lowest curve, we suppose that Na^+ and Cl^- are brought close to each other adiabatically. At

$$\text{large distances } F(r) = -\frac{\delta U}{\delta r} = -\frac{e^2 k}{r^2}$$

where k is a proportionality factor. When they come very close, mutual polarisation of the ions sets in, which gives rise to repulsive forces of the type $\frac{\mu}{r^n}$ ($n = 5$ in this case).

U has therefore the value :

$$-\frac{e^2 k}{r} + \frac{\mu}{r^5} + C$$

and it has a minimum value at the point where $\frac{\delta U}{\delta r} = 0$.

The minimum value of U is taken to be zero. This is the point A_0 . The dotted line is asymptotic to the $[U; -r]$ curve for Na^+ and Cl^- , and the intercept D_i represents the heat of dissociation of NaCl vapour into Na^+ and Cl^- . We may call D_i the heat of ionic dissociation.

The curve II represents the variation of U with ' r ,' when the constituent particles are neutral Na and neutral Cl. At large distances, the force, being entirely between neutral atoms, becomes negligible, hence U is pretty nearly constant. The Ur curve is supposed to have no minimum value and rises abruptly upwards with decreasing value of r . The intercepts represent the heat of dissociation into Na and Cl. In the case of NaCl, $D_a = 109$ k.cal., whereas $D_i = 137$ k.cal. Hence, $D_a < D_i$.

The continuous absorption is explained by the line A_0B . We suppose the molecule to be in the state A_0 . When light falls on the electrons of Cl^- it passes over to Na^+ , and the combination is NaCl; the representative point is, therefore, B, as we suppose the distance to remain unchanged. As B is above the asymptotic line D_a , the molecule breaks up. We have, then,

$$h\nu_0 = D_a.$$

If the vapour is sufficiently heated, we may have other vibration states in sufficient numbers, marked by the representative points $A_1, A_2, A_3 \dots$. In these cases, the transitions will be A_1B_1, A_2B_2 , etc. The limit of absorption will shift towards the red, as was first discovered by Evans,⁴ and investigated by Sommermeyer⁵ in detail. Sommermeyer observed the fine structure beginning of continuous absorption, and from this calculated the distances A_0A_1, A_1A_2 , etc., giving a sequence of values of ω_n . From this, we have, using Birge and Sponer's method :

$$D_i = \int_0^{\infty} \omega_n dn$$

This D_i ought to coincide with the value of D_i obtained from other sources. This does not seem to have been attempted. We have :

$$D_i = D_a + I_{Na} - E_{Cl}$$

where I_{Na} = heat of ionisation of Na, E_{Cl} = electron affinity of Cl.

It was found by Franck and Kuhn,¹ that the absorption spectra of silver halides show, in addition to the con-

tinuous absorption, a system of discontinuous bands shaded towards the red. From a number of arguments which are summarised below, Franck was led to the hypothesis that in silver halides the binding is not of the ionic type as in NaCl, but of the atomic type. Brice¹ investigated the absorption spectrum of AgCl with greater dispersion and could identify the bands due to the different isotopes. His investigation throws no clearer light on the nature of binding, except rendering some of Franck's conclusions doubtful. In the following we shall attempt an alternative explanation of the experimental results of Franck and Kuhn and that of Brice.

The arguments of Franck and Kuhn regarding the nature of binding in silver halides are summarised below :

(1) The discontinuous absorption of AgI and other halides consists of a set of bands shaded towards the red as in non-polar molecules like I_2 . A large number of vibration terms in the normal state as well as in the excited states have been calculated. From this it has been found, by applying the Birge and Sponer's method,⁶ that for AgI the value of the heat of dissociation is 2.0 volts. In the following table we have given the values of the heats of dissociation as given by Franck, calculated thermochemically and from optical data.

	Heat of dissociation calculated by thermal methods	Heat of dissociation calculated optically
AgCl	3.11 volts	3.13 volts
AgBr	2.6 „	2.4 „
AgI	2.1 „	2.0 „

From the equality of the two values, Franck concludes that the normal molecule, when allowed to vibrate, will dissociate into atomic Na and atomic Cl. With regard to this conclusion, we may point out here that the agreement is entirely fallacious, as Franck's values for the atomic heat of formation are based on the assumption that λ_0 (latent heat of evaporation of silver at absolute zero) = 55.8 k.cal. It seems to have been taken from Landolt and Bornstein's Tables, p. 1922 of 1923 edition.

But recently λ_0 for Ag has been very carefully calculated by Harteck,⁷ who finds that the value is 72 k.cal. We have, therefore, recalculated the atomic heat of formation by using a Born cycle. We have :

$$R = \lambda_0 + Q + \frac{1}{2}D_{Cl_2} - \lambda_{AgCl}$$

λ_{AgCl} = latent heat of vaporisation of the halide. The recalculated values in k.cal. are shown in the following table :

	λ_{AgCl}	$\frac{1}{2}D_{hal}$	Q	R	$\lambda_{Cal.}$	λ_{Obs}	Franck's value of R
AgCl	44.2	28.3	31	88	3250	2790	70
AgBr	40.8	23.3	27.5	82	3510	3450	61
AgI	36.8	18.0	22.5	74	3860	3710	46

In the above table λ_{AgCl} , etc., have been calculated from the vapour pressure measurements of Jellinek.⁸ 'Q' has been taken from the *International Critical Tables*, vol. 5, p. 188. In the case of bromides and iodides, we have added to the given values half the molar heat of sublimation of Br (liq.) and I (solid), as the figures in the tables correspond to the formation out of the natural states of these substances, whereas we require the heats of formation from the gaseous state.

It is seen from comparison of columns 5 and 8 of the above table that the value of R diverges widely from the value obtained by Franck, using Birge and Spomer's method.

(2) AgBr is found to give a continuous absorption beginning from λ 3450, corresponding to $R = 83$; as this did not agree with Franck's value of R (61), he assumed that the photochemical action corresponds to the dissociation of AgBr into Ag and Br₂, which gives $R = 70$ k.cal. But this corresponds to $\lambda = 4000$ Å. The assumed agreement thus seems to have been rather forced.

A comparison of columns 6 and 7 shows that the discrepancy still remains, but it is not absolutely necessary that λ calculated from R should always tally with λ_{Obs} . All that we can say is that λ_{Obs} should always be less than $\lambda_{Cal.}$, unless careful microphotometric measurements are done, which is impossible in the present case, due to superimposed bands.

Franck lays great stress on the fact that there is only one absorption maximum in the case of the bromide and the iodide, whereas according to analogy of alkali halides, there ought to be at least two maxima. This point ought to be further investigated, but we may mention that the continuous absorption in these cases is, according to our experience, always masked by band absorption on the short wavelength side. We shall see later, that in this case the probability of the existence of the second maximum according to the excitation of Br or I from $^2P_{\frac{3}{2}}$ to $^2P_{\frac{1}{2}}$ is rather remote.

Brice at first contended that the lower state is atomic and the excited molecular state giving the bands corresponded to both the atoms being in the excited states. Due to large discrepancy in the energy relations, he had later on to reject this hypothesis. Since the curve corresponding to continuous spectrum must be atomic, the only alternative that remains is that both the configuration states are ionic.

The electrical conductivity data of these compounds in the fused states, as well as the crystal structure in the solid state, are decidedly against Franck's hypothesis. Crystals of AgCl and AgBr are ionic aggregates just like NaCl and NaBr.

Franck states, further, that values of ω (the fundamental frequency of nuclear vibration) do not change from the normal to the excited states, and from this he concludes that the type of binding remains unaltered in the two states. They can, however, be both atomic or both ionic. Franck decides in favour of the former view, but, as discussed above, his arguments cannot be justified. We are of opinion that the binding is ionic both in the normal as well as in the excited states. In the normal state the composition is Ag^+Cl^- , the constitution of Ag^+ being $4d^{10}$. In the excited state as well, it is Ag_e^+Cl^- , Ag_e^+ has got the electronic constitution as $4d^9 5s$. The continuous absorption is due, as in the case of NaCl, to the decomposition of AgCl into Ag (neutral) and Cl (neutral). The hypothesis is illustrated in the following figure for the various states.

It will be noticed that the forms of the curves I and II

are the same, as they are both of the polar type. The value of ' r ' has been taken to be very nearly the same in both the curves. The curve III for AgCl has been taken to be flat in its upper course, as in the case of NaCl. From the curves I and II the intensity relations of the bands of AgCl, as well as of AgBr and AgI, can be explained. It has been found by Brice that the strongest bands correspond to the transition O-O. This is in agreement with our diagram,

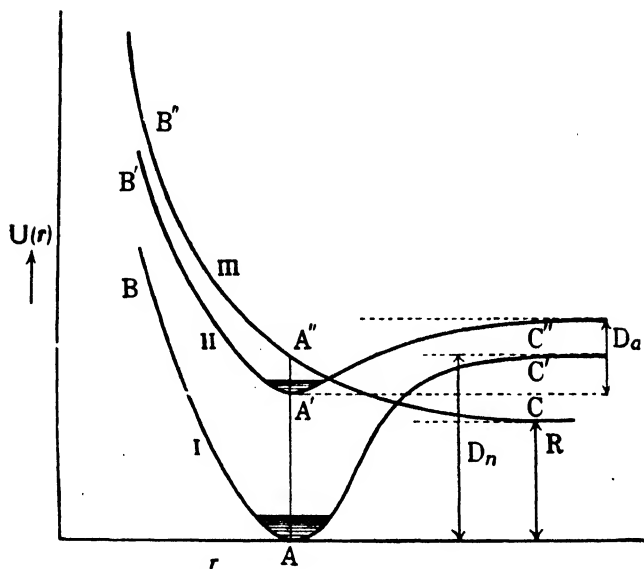


FIG. 131. Potential energy curves of AgCl in different states.

as according to Franck's hypothesis of slow change of the nuclear distance the strongest transition would correspond to the transition denoted by the perpendicular line from the minimum point of the lower curve.

Curve III in the figure represents the $U(r)$ diagram for Ag and Cl. The binding is nonpolar and according to Franck should be of type II in fig. 130. But the departure of observed values from the values of R is explained if we assume that the $U-r$ curve for AgCl has the form as shown in III, fig. 130. Such forms have been postulated by Herzberg⁹ in the case of the methyl halides. But of course,

at present, we do not know much of these types of curves excepting in the special case of H_2 , worked out by Heitler and London¹⁰ and by others.

If the above view be correct, then it will be possible to find out the heat of ionic dissociation of $AgCl$ molecules into Ag^+ and Cl^- from the following relations :

$$D_i = R + I_{Ag} - E_{Cl}.$$

where I_{Ag} = ionisation potential of silver, E_{Cl} is the electron affinity of Cl .

$$D'_i = R + I_{Ag} - E_{Cl} + E_{Ag} - h\nu_0$$

where E_{Ag} is the energy of excitation of Ag^+ from $(4d)^{10}$ state to $(4d)^9 5s$ state. This energy is not yet precisely known. It is variously given between 2.8 volts and 4.8 volts. $h\nu_0$ is the energy of excitation of the O-O band ; it has been found to be 3.9 volts. Since $I_{Ag} = 7.53$ volts, $E_{Cl} = 3.9$ volts, we get,

$$D_i = 7.51 \text{ volts}$$

$$D'_i = 3.61 \text{ volts} + E_{Ag}$$

hence $D'_i > 6.4$ and < 8.4 volts.

Brice¹ finds these values, with the help of Birge and Sponer's method, to be 3.11 volts and 0.31 volt respectively. Hence they are too small and, if correct, invalidate the theory put forward in this article.

But no positive proof has yet been given that Birge and Sponer's method of calculating the heat of dissociation from a sequence of vibrational terms ever applies to polar compounds. Even in the case of non-polar compounds it holds rather poorly in most cases, particularly when the convergence of ω_n values is rather slow.

It must be admitted, however, that no definite and positive assertion can be made with regard to the nature of binding in silver halides. Franck propounded a view which could not stand scrutiny. Brice tried and failed to put forth a successful picture of the whole. An alternative suggestion has been put forth here which is supported from various considerations, as detailed above. The results

which have been given appear to contradict the energy relations as calculated by Birge and Spomer's method. This aspect of the subject requires further investigation, which we expect to undertake in future.

SUMMARY

In this paper a view has been put forward that the molecules of silver halides in the vapour state form ionic compounds and not atom compounds as postulated by Franck. The normal state is considered to be of the constitution Ag^+Cl^- , where Ag^+ is in the $(4d)^{10}$ state. The excited state is also given by Ag_e^+Cl^- , but Ag_e^+ is now in the $(4d)^9 5s$ state.

The $[U - r]$ curve for the neutral combination, AgCl , has a hyperbolic shape, which accounts for the fact that $h\nu_0$ (ν_0 = limit of absorption frequency) is $> R$, the atomic heat of dissociation. Arguments for and against the two views are discussed.

In conclusion, I wish to express my grateful thanks to Sir J. C. Bose, F.R.S., for the encouragement and facilities offered to me at the Bose Research Institute for carrying out this investigation. I also take this opportunity to express my indebtedness to Prof. M. N. Saha, F.R.S., for discussion and helpful criticism of the paper. My thanks are also due to Dr. S. C. Deb for allowing me to utilise some of his data in this connection.

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XIV.—ABSORPTION SPECTRA OF LEAD HALIDE VAPOUR

BY

SURESH CHANDRA DEB, D.Sc.

It has long been known that by studying the nature and structure of spectra, both in emission as well as in absorption, it is possible to get an intimate knowledge of the constitution and the strength of binding of molecules. But while the emission spectrum, on account of its complex nature, is rather difficult to interpret, the case of absorption spectra is much simpler. Here, as the initial level is always the same, the spectrum is comparatively simple, and consequently the interpretation is easier. There is one more advantage in the study of molecular spectra in absorption. To excite the emission spectrum electrical discharge is allowed to pass through the gas, and generally in this process the molecule, particularly if it is a polynuclear one, is broken up, and it is rather difficult to say whether the spectrum observed is due to the molecule or due to some decomposition product of it. Such difficulty is not encountered in the case of absorption spectra, which can generally be assigned to the undecomposed molecule.

The absorption spectra due to molecules can be grouped under several headings. There are a large number of molecules, *e.g.* alkali halides, that do not show a trace of band absorption in the visible and in the ultraviolet part of the spectrum, but only a continuous absorption beginning from a long wavelength limit is usually obtained. Franck and his co-workers¹ showed that in the case of alkali halides this limit corresponds to the breaking up of the

molecule into neutral atoms, and further that the thermal heat of dissociation of the molecule into atoms and the energy of dissociation calculated from the beginning of absorption become identical within experimental error. It was observed that mostly the molecules that were classified as ionic in chemistry show this continuous absorption, and the non-ionic or atomic molecules show band absorption. This led Franck² to group the molecules into two classes: (a) ionic or heteropolar molecules, and (b) atomic or non-polar molecules. Subsequently, Mulliken³ sought to generalise the classification by saying that molecules with unsaturated valency show band absorption while those with saturated valency show continuous absorption.

After these views were put forward, a large amount of experimental work on absorption by molecules was performed at the Physical Laboratory of the Allahabad University, at the Molecular Physical Laboratory in Leningrad and at Franck's Laboratory at Göttingen, which has led to considerable modification of the above views. The different types so far observed can be grouped under the following headings:

A. Saturated molecules.

- (i) Those which show continuous absorption and obey Franck's rule (*e.g.* alkali halides).
- (ii) Those which show band absorption along with continuous absorption on account of the presence of metastable levels in the metallic constituent (*e.g.* alkaline earth halides, silver halides, and halides of the transitional group).
- (iii) Those which show only continuous absorption and apparently disobey Franck's rule (*e.g.* hydrogen halides).

B. Recently a new type of continuous absorption has been obtained in di-halides of the fourth group of elements which await explanation.

Prima facie, it is difficult to say whether this class of compounds is ordinarily to be regarded as saturated or not. The highest valency of this group of atoms is usually four. Thus for Pb the saturated chloride is PbCl_4 , but PbCl_2 is also fairly stable. The oxides and sulphides of this group can be treated from the same point of view, and here also we have a dioxide and a monoxide. Thus for Pb, PbO_2 is the saturated compound, while PbO is apparently unsaturated. Are we to regard such compounds as saturated or unsaturated? Let us see what light is thrown from the study of their absorption spectra.

Experiments which are going to be described indicate that these apparently unsaturated molecules show only continuous absorption with more than one cut in the visible and the quartz regions. Some of these cuts can be interpreted, though with difficulty, by using Franck's law regarding alkali halides. It may be noticed in this connection that though carbon, silicon, lead, etc., belong to the same group and have identical constitution in the outer shell of electrons they show wide divergence in the case of their molecular formation. Thus no carbon-di-halide is known corresponding to Pb or Sn di-halides, which are stable compounds. In the case of oxides there exists a carbon monoxide corresponding to PbO and SnO , but the nature of binding is probably quite different as CO shows band absorption and no continuous absorption has yet been discovered, whereas in the case of the monoxides of lead and tin Mr. P. K. Sen Gupta⁴ finds that absorption is perfectly continuous. As far as sulphides are concerned, no stable mono-sulphide of carbon is known, though we have very stable compounds in PbS and SnS . These cases indicate that compound formation is not merely a matter of the constitution of outer electron shells as is sometimes believed by certain authors, but, on the other hand, is a very complex phenomenon which can be elucidated only if such diverse cases are studied from as many points of view as possible. In the present investigation, the results of my study of Pb di-halides are reported. These results are of a rather baffling nature, and though

I have tried to give explanations it appears that many more similar cases must be studied before any final conclusion can be drawn.

EXPERIMENT

Vapour pressures of these compounds have, fortunately, been studied by a number of workers. From their work we find that to obtain sufficient vapour, rather high temperature is necessary, viz., for PbCl_2 a temperature of 900°C. , for PbBr_2 a temperature of 800°C. , and for PbI_2 750°C. are required to produce saturated vapour pressure of one atmosphere. Hence for all these compounds the vacuum graphite furnace of the laboratory was used. As the description of the furnace and its workings have been published in a number of papers previously,⁵ they are not further repeated here. The furnace could be used in vacuum, but it is better to fill it with nitrogen or with some other inert gas. The gas checks rapid evaporation and diffusion of the substance under investigation. In the present case the absorption experiments were performed by filling the chamber with nitrogen.

The source of continuous light was an under-water spark through two copper electrodes. The light from the spark after being rendered parallel with a quartz lens was allowed to pass over the hot vapour of the lead salts and then photographed with an E_3 -Hilger quartz spectrograph. Pressure inside the furnace was controlled by the temperature of the furnace, which could be manipulated as desired. A copper arc served the purpose of comparison spectrum.

RESULTS

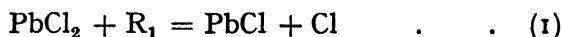
All these photographs showed only continuous absorption beginning from a long wavelength limit. Three retransmissions in the case of chloride and two each in the case of bromide and iodide of Pb were found. Details are given in the following table.

TABLE I.

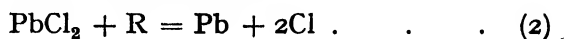
	First cut		Second cut		Third cut	
	λ	k.cal.	λ	k.cal.	λ	k.cal.
PbCl ₂	3860	74.1	3420	83.63	2960	96.62
PbBr ₂	—	—	4580	62.44	3300	86.66
PbI ₂	—	—	5780	49.48	2580	110.85

CALCULATIONS

The ideas put forward in the beginning of this paper are now applied to the molecules under investigation to see how far they are similar to perfect ionic molecules like alkali halides. Let us assume that the primary products of dissociation of PbHa₂ are PbHa and Ha (Ha meaning halogen), and the energy to bring about this dissociation is R₁ k.cal. Taking the particular case of PbCl₂, this is



The final process may be taken to be



This R can be determined with the aid of the following thermochemical relation :

$$R = Q + D_{\text{Cl}_2} + L_{\text{Pb}} - L_{\text{PbCl}_2} \quad . \quad . \quad (3)$$

where Q = heat of formation of PbCl₂ out of [Pb] and Cl₂

L_{Pb} = latent heat of vaporisation of Pb

L_{PbCl₂} = latent heat of vaporisation of PbCl₂

D_{Cl₂} = heat of dissociation of Cl₂.

All these quantities are from the natural state of the substances.

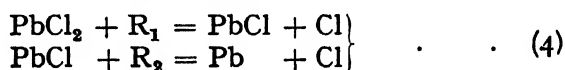
In the following table (Table II) will be found the numerical data for all these quantities in k.cal. The values

of R for PbCl_2 , PbBr_2 and PbI_2 molecules, as calculated with the help of the formula (3) above, are also included in the table. The latest value of the latent heat of lead is determined by Harteck ⁶ and is given as 48 k.cal.

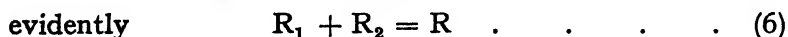
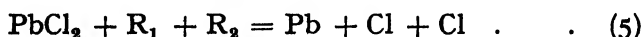
TABLE II.

	Q'	L_{PbHa} ⁸	D_{Ha} ⁹	R
PbCl_2	86	33.5	56.8	157.3
PbBr_2	66.5	33.5	45.2	126.2
PbI_2	42	32.3	35.5	93.2

Comparing Tables I and II, the following photochemical processes to account for the observed cuts can be postulated :



i.e.



It is difficult to determine R_1 and R_2 separately by an unambiguous method. According to Datta and Saha,¹⁰

$$R_1 = R_2 = R/2 \quad (7)$$

The position of the second cut is very nearly given by $R/2$. Thus for

$$\text{PbCl}_2 \quad R/R_1 = 157.3/83.63 = 1.88$$

$$\text{PbBr}_2 \quad R/R_1 = 126.2/62.44 = 2.02$$

$$\text{PbI}_2 \quad R/R_1 = 93.2/49.48 = 1.89$$

But according to this view it is difficult to interpret the other cuts.

Table I indicates that both PbBr_2 and PbI_2 should show cuts corresponding to the first cut of PbCl_2 . The

experiments could not be continued sufficiently for testing these points, but they will be resumed.

Popov and Neujmin¹¹ have tried to calculate the value of R_2 , viz. the heat of dissociation of $PbCl$ into Pb and Cl by two different methods, none of which seem to be entirely satisfactory. In one method they have intrapolated the value of D_{PbH_2} (heat of dissociation of $PbCl$ into Pb and Cl) from corresponding values of D for $ThCl$, $AgCl$ and $HgCl$, plotted against their respective ionisation potential as shown in the following Table III.

TABLE III.

	Th	Ag	Hg	Pb	Na	Li
I.P.	140.2	173.8	239.6	170.2	117	124
Cl	87.0	71.7	30.0	73.1	108	118
Br	73.5	63.9	25.0	64.5	84	109
I	55.0	47.0	15.0	45.7	63	78

The values in column 5 are extrapolated. There seems to be very little theoretical justification for such a procedure, as the type of binding is entirely different in the different cases.

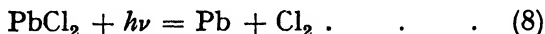
DISCUSSION OF POPOV AND NEUJMIN'S EXPERIMENTS

It thus appears that the result so far obtained is insufficient to explain the nature of binding in molecules of the type $PbCl_2$. No further light is thrown by the experiments of Popov and Neujmin above referred to. These authors subjected $PbCl_2$ vapour heated to about $400^\circ C$. (vapour pressure 0.002 mm.) to light from Zn , Al and Fe sparks, and examined the fluorescent spectrum. We shall

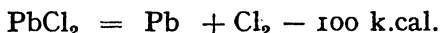
discuss the results they obtained with Zn spark λ 2066—having an excitation voltage of 6.67 volts. Their results are as follows :

- (a) Excitation of the bands of Cl_2 from λ 5619 to λ 4941. The excitation voltage for this is 2.5 volts.
- (b) Excitation of bands due to PbCl from λ 4098.5 to λ 4921.4; corresponding excitation voltage is 3.01 volts.
- (c) Excitation of the lead line λ 4058. This requires an excitation voltage of 5.68 volts.

The Cl_2 bands are excited in some strength. It may be remembered that with pure chlorine, in contrast to iodine, it is very difficult to get either absorption or fluorescence. This is due to the fact that as electron transition is $^2P_{3/2} \rightarrow ^2P_{1/2}$ and as the intensity is proportional to ν^4 , *i.e.* to $\nu_{(^2P_{3/2} - ^2P_{1/2})}^4$ this is very small in Cl_2 owing to the small value of $\Delta\nu(^2P_{3/2} - ^2P_{1/2})$.¹² But in the case of PbCl_2 the Cl_2 bands are excited rather strongly. Hence Popov and Neujmin are at first inclined to ascribe it to a process :

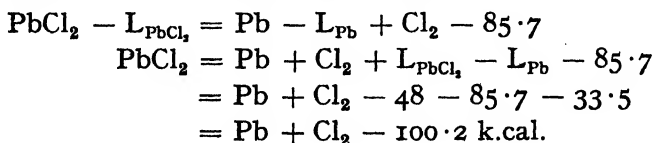


i.e. the incident light causes decomposition of PbCl_2 into normal Pb and Cl_2 excited (denoted by Cl_2^*). But this explanation fails on considerations of energy :

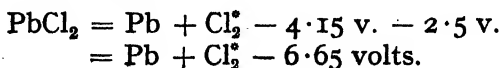


because $[\text{PbCl}_2] = [\text{Pb}] + \text{Cl}_2 - 85.7 \text{ k.cal.}$

and from thermochemical measurements



Hence we must have



But the energy of exciting light from Zn spark λ 2066 is only 6.09 volts. Hence the exciting quanta have too little energy to cause this process. Popov and Neujmin reject this process on other grounds, viz. that as chlorine is presumably situated on two sides of Pb, one quantum cannot simultaneously detach both Cl's and cause formation of Cl_2^* excited.

The other two possible processes

$\text{PbCl}_2 + h\nu_1 = \text{Pb (excited to give } \lambda \text{ 4058)} + \text{Cl}_2$
and

$\text{PbCl}_2 + h\nu_2 = \text{PbCl (excited)} + \text{Cl}$

are also improbable on the same energy considerations.

Popov and Neujmin think that at the temperature of 500°C . PbCl_2 is thermally decomposed into Pb and Cl_2 and PbCl and Cl, and then the individual particles are excited by the incident light. But it is improbable that at such low temperatures PbCl_2 can be decomposed in any appreciable quantity. Calculations on this point, based on theories of thermal dissociations, will be given later. As far as Cl_2 bands are concerned, this explanation cannot account for their great strength, for we are, after all, reduced to excitation of pure Cl_2 by light. On this point Popov and Neujmin seem to be arguing in a circle.

SUMMARY AND CONCLUSION

In this paper the absorption spectra of lead halides have been studied. It has been shown that, though apparently they are unsaturated, they show the characteristics of saturated molecules, inasmuch as absolutely continuous absorption, beginning from a long wavelength limit, is obtained. Thus the criteria to decide between saturated and unsaturated compounds become controversial, and an attempt has been made to clarify the position in a short discussion. The results of the experiments have been interpreted in the light of Datta and Saha's suggestion regarding the photodissociation of polyatomic molecules. In this connection the interpretation of some experimental

results and the validity of certain other conclusions as given by Popov and Neujmin have been discussed in detail. Finally, the necessity of some further experiments which I propose to carry on in this Institute has been indicated.

In conclusion, I wish to express my grateful thanks to Sir J. C. Bose, F.R.S., for the encouragement and facilities offered to me at the Bose Research Institute in carrying out this investigation. I also take this opportunity to express my deep sense of gratitude to Prof. M. N. Saha, D.Sc., F.R.S., for allowing me to utilise some experimental material from his laboratory, and for helpful criticism of the paper.

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